

Chemistry 426

Capillary Gas Chromatography - Mass Spectrometry

Introduction

The most powerful tool in the analytical laboratory for the characterization of complex mixtures of organic molecules is the capillary gas chromatograph-mass spectrometer or GCMS. Its extraordinary capabilities are attained by separating the components of a mixture using capillary gas chromatography, a high resolution chromatographic technique and 'fingerprinting' each component in real time as it leaves the chromatographic column with a rapid scan mass spectrometer. An essential element of this instrument is a computer with a graphics display terminal and a large amount of mass storage. In addition to controlling the operating parameters of the gas chromatograph and the mass spectrometer, the data system allows the progress of the analysis to be monitored as it takes place, writes all of the data to permanent storage on disk, and provides a variety of routines for post run analysis of the data.

Data Acquisition

Double click with the left mouse button on the GC_MS Instrument #1 icon on the computer monitor. Note the activity on the bottom left of the screen as the instrument control software is loaded into memory. When **Welcome to Instrument Control** appears on the bottom of the screen the instrument controls will function.

Click on the **Instrument** tab then click on **Perform MS Auto Tune**. The instrument will undergo a self tuning process to detect mass to charge ratios correctly.

When the tuning is complete a tune report will print out.

Next click on the **View** tab and select **Top**.

Click the **Sequence** tab and select **Load**.

A browser window will open. On the right screen in the browser select the following directory tree: c:\hpchem\1\sequence\.

From the left screen select the Chem426.s file and click the **OK** button. The browser window will close.

Click the **Sequence** tab, and select **Edit Sample Log Table**.

You will notice that AIR, STANDARD, and SAMPLE are located in vial positions 1, 2, and 3. You will be given three vials labeled air, standard, and sample. Place these vials in their correct positions in the sample carousel. If sample vials are already in those positions notify the TA, who will remove them and replace them correctly after the lab.

Click **Cancel** on the Log Table.

Click the **Sequence** tab and select **Load and Run Sequence**.

The **Load Sequence** browser window will open.

Select Chem426 from the left window and click **OK**

Check **Setup** and **OK**.

The **Start Sequence** window will open.

Under **Data File Directory** verify that the path:

D:\USERFT~1\USERS\CHEM426\

is present. There may be another directory name after the CHEM426. If there is, replace it with a directory named with today's date.

Select **Run Sequence**.

Allow the instrument to operate. Samples will be injected automatically and chromatograms will appear on the screen. When the analyses are complete click on the x in the upper right corner of the screen to end the Chem Station Session.

Data Analysis

With the left mouse button double click on the Data Analysis #1 icon on the screen.

Click on the **File** tab and select **Load Data File**.

A browser window will open. On the bottom of the window select the d: drive.

Today's Chem 426 data is in the path:

d:\USERS~1\USERS\CHEM426\'Today's Date\

Dead Time

Select the Air.d data file.

From the **File** tab select **Load Method**.

Note: For any messages that appear while loading a method, click either yes or OK and continue.

From the browser select the path and method file:

c:\hpchem\1\Mehtods\air.m

From the **Int.** tab select **Ion Chromatograms**
An **Extracted Ion Chromatograms** window will appear.

Leave the **Time Range** as it is.

In the Ion boxes enter 28 for #1, 32 for #2, and 19 for #3. Nitrogen has a m/z ratio of 28, oxygen 32, and 19 doesn't correspond to anything so it shouldn't give any response and serve as a null test.

Use a **Mass Range** from 0.30 to 0.70.

Click **OK**

Three plots appear, one for each mass selected.

Under the **Int.** tab select **Integrate**.

The time of each peak, where there is a peak, will appear above it.

Select **Print Trace + Spectrum**

Quantitative and Qualitative Analysis on Selected Ions

Under **File** select **Load Data File** and select the Standard.d file under the same path as the Air.d file.

Under **File** select **Load Method** and select the Chem426 method from the same path as the air method.

Under the **Lib** tab select **Generate Lib. Search Report**.

The program will search the NIST (National Institute of Standards and Technology) library of fragmentation patterns to find the best matches for the peaks present in the standard.

Print the report to obtain a list of components in the standard with their retention times.

Under the **Int.** tab deselect **Integrate a peak** (it should not have a check before it).

If there was a cross hair on the chromatogram plot it should have changed to a vertical line. Place the cursor to the left of the first peak. Hold down the right mouse button and drag the cursor across the peak. When the mouse button is released the fragmentation pattern of the analyte making up the first peak should appear on the lower screen with the m/z ratio above the larger fragment peaks. The height of the fragment peak indicates the abundance of that fragment as the molecule fragments in the detector. On the Library Search Report record the m/z values of parent ion (the fragment with the highest m/z ratio) and the next most abundant fragment. Create fragmentation patterns for the remaining peaks on the upper plot and record the parent ion m/z ratio along with the next most abundant m/z ratio.

Under the **Quant** tab select **Qedit Quant Results**

A popup window will appear with a list of the compounds identified in the standard. Click on Start and the analytes in the standard will be updated. When each compound is finished being highlighted click **Stop** then **Exit**.

Respond Yes to Save Changes.

Under the **Init.Cal.** tab select **Update Levels**

Select **Recalibrate** in the first pull down window.

Set **Responses** to **Replace**

Set **Retention Times** to **Replace**

Click **Do Update**

Select **View** and go through each compound in the standard. The previous and next page buttons on the bottom of the popup window can be used. The **Tgt.** Square should contain the parent ion m/z ratio. The **Q1** should contain the most abundant m/z ratio that is not the parent ion.

Click **Next** to go to the next compound.

Click OK after checking all **Tgt** and **Q1** entries. Click **OK** then exit.

Under **File** select **Load Data** and load the **sample.d** file.

Under the **Quant** tab select **Calc/Gen Report**.

Use the pull down window in the popup to select **Detailed**

Deselect the printer so there is no **x** in the little box by **Printer**.

Click **OK**

Under the **Quant** tab select **Edit Quant Report Options**
Click **Report** on the popup window. Then **Detailed**, then **OK**

A detailed quantitative report of the analytes in the sample will print.

Pre-Lab Questions (10 pts.)

1. Draw a schematic diagram of the GC and MS, and label each component, including the three basic components of the mass spectrometer.
2. Column head pressure, split ratio, and temperature are important operating parameters. Briefly discuss their significance to GC operation.

Data Presentation (30 pts.)

3. Report the retention time and identity of each compound quantitated.
4. Provide the total ion chromatograms for the standard and the sample runs
5. Circle the parent ion peaks and qualitative ion peaks in the fragmentation patterns that appear with each analyte in the sample.
6. Solutions are made in mg / mL. Report the concentration in the sample that were included in the standard except bromobenzene Include the quantitation printout of these compounds.

Post-Lab Questions (10 pts)

7. Why would a laboratory build their own mass spectral library when large libraries are available commercially?
8. What are the benefits of coupling a mass spectrometer to a GC, rather than using a standard GC detector like a thermal conductivity detector or a flame ionization detector?

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Fluorescence Measurements

Prelab Questions:

10 pts.

1. Which has more energy a photon with a wavelength of 350 nm or a photon with a wavelength of 250 nm.
2. True or False: A molecule at room temperature can create energy and release a quantum of energy higher (i.e. at a shorter wavelength) than it absorbs?
3. If a molecule is excited by light at 350 nm could it emit light at 250 nm?
4. When single wavelength of light exits a slit from a light source and hits a sample cuvette that light can be reflected and refracted in many directions. To avoid huge emission peaks caused by light from the source, what precaution must be used in the emission wavelength selection criteria.

The Utility of Fluorescence

Fluorophores or fluorescing molecules can become electronically excited by the absorption of light. When a single electron is excited out of the ground state and leaves behind a paired electron of the opposite spin, it can emit a fluorescent photon to return to ground state. Because it is returning to an orbital with another electron of paired (opposite) spin, the process can occur rapidly in a period or approximately 10 ns. Emission spectroscopy is more sensitive than absorption spectroscopy because the emission signal is compared electronically with a reference emission of zero, whereas absorption spectroscopy compares a difference in the intensity of two quite high energy beams and a weakly-absorbing signal may be lost in the instrument noise.

The Perkin Elmer LS-50B Fluorimeter will be used to study emission spectroscopy and investigate how fluorescence is affected by concentration of fluorophores and their quenching agents.

Ruthenium and quenching.

Tris(2,2'-bipyridine)ruthenium (II) chloride $[\text{Ru}(\text{bipy})_3^{2+}]$ will be used as a source of ruthenium in this experiment. Consider the absorption of a photon by the Ru^{2+} ion represented below.

$\text{Ru}^{2+} + h\nu_0 \rightarrow \text{Ru}^{2+*}$, where ν_0 represents a photon absorbed in the UV region and Ru^{2+*} represents the excited state of the ruthenium ion. If light is constantly impinging on the sample this reaction would be ongoing at a constant rate.

This process can then be reversed by changes in the vibrational state and release of heat or nonradiative energy of the molecule and release of a lower energy photon ν .

$\text{Ru}^{2+*} \rightarrow \text{Ru}^{2+} + h\nu$. The rate constant of the reaction is represented as k_s .

If the electron involved in the release of the photon were to be stolen from the ruthenium atom by a nearby metal ion, M, then it would not be available to release a photon as fluorescence and the fluorescence would be quenched. That reaction could be represented as:

$\text{Ru}^{2+*} + \text{M}^{(n)+} \rightarrow \text{Ru}^{3+} \text{M}^{(n-1)+}$ The rate constant of this reaction is represented as k_q .

These last two reactions compete and the intensity of the fluorescence of the quenched reaction is represented by the Stern-Volmer equation:

$$I_0/I = 1 + [\text{M}^{n+}] k_q / k_s.$$

The derivation of this equation is not within the scope of this course but students can refer to reference 2 for further information. However as analytical chemists we will all be delighted that the equation takes the form of a straight line. I_0 represents the emission intensity with no quenching metal present. I is the emission intensity when the concentration $[\text{M}^{n+}]$ of quenching metal is present in solution. The spontaneous decay of $\text{Ru}(\text{bipy})_3^{2+}$ has been studied and its rate constant, k_s , is 1.67×10^6 .

The quenching constant k_q depends on the particular metal. As can be seen by the charge transfer from the ruthenium to the metal a redox reaction occurs. k_s also depends on the ionic strength of the solution as well as the temperature. By measuring the emission intensity of $\text{Ru}(\text{bipy})_3^{2+}$ in the presence of multiple concentrations of quenching metal under constant conditions of temperature and ionic strength a Stern-Volmer plot will yield a line of slope k_q / k_s . From this the rate constant of the quenching reaction will be determined.

General Operating Instructions for the Emission Analysis using the Perkin Elmer Model LS50B Luminescence Spectrometer

Specific instructions will follow for analyzing samples in this experiment.

Wear gloves while handling solutions for this lab. Rinse the fluorescence cuvette provided with ethanol from a wash bottle to remove any contamination from previous use. Collect the waste in the waste bottle for this experiment.

- 1) Verify that the LS50B is turned on as indicated by the small red light in the front of the instrument.
- 2) Turn on the Computer Monitor and double click on the *Fluorimeter* icon.
- 3) Let the TA set up a folder from the **Utilities – Configuration – Data** options for data collected at today's date, so it won't be stored in someone else's folder.
- 4) Double click on the *Chem426.mth* line.
- 5) Click on the *set up parameters* tab.
- 6) Click on the *Emission* tab.

In the *Start nm* window enter a value larger than the maximum peak wavelength from the absorbance scan and *End nm* windows enter a wavelength near 900nm. When measuring emission intensities near the excitation wavelength, reflectance from the surface of the sample cuvette and refraction and scattering from within the sample itself will cause very high readings to appear, so all emission measurements must start far enough above the excitation wavelength to avoid collection of reflectance intensities that will cause the auto-scaling feature of the plot screen to make the emission data unreadable.

- 7) In the *Excitation nm* window enter the wavelength of the maximum peak from the absorbance.
- 8) Enter 10.0 in the *Exit Slit(nm)* and *Em Slit(nm)* windows.
- 9) Set the scan speed to 200 nm per minute.
- 10) On the *Results Filename* window enter a file name named after the date followed by a letter. Then click the *Auto increment filenames* box and each new spectrum file name will have a number one larger than the previous file name added to it.
- 11) Click on the *Traffic Light* icon in the upper left if it is green. If it isn't green ask the TA for help.
- 12) Click the *View Results* tab.
- 13) While the first spectrum is being acquired, pass the cursor over the icons on top of the chart and read the pop up balloons to see what they do.
- 14) After the entire spectrum is displayed and the *Traffic Light* icon returns to green autoexpand the ordinate axis and print the graph using the print icon. The printer is located behind and to the left of the instrument as you face it.
- 15) When instructed to measure the emission at a fixed wavelength while varying the excitation wavelength, click on the excitation tab. Set the *Start nm* near 200 nm and *End nm* to a value 10 nm less than the emission wavelength. Set the *Emission* wavelength to the wavelength where a maximum emission peak was seen on the scan where the emission wavelengths were varied.
- 16) Again click on the green *Traffic Light* icon and go to the *View Results* tab.

Experimental:

The solvent for this experiment is 0.5 M H₂SO₄. Sulfuric acid fluoresces in the UV range. Plastic cuvettes absorb low UV radiation and can reduce the amount of radiation entering in the low UV to excite the sulfuric acid. The ruthenium compound in this case absorbs a fair amount of radiation above the absorbing maximum of the plastic cuvettes.

Absorption Spectra:

For this experiment an air blank will first be scanned on the UV-VIS spectrometer using the wavelength range 190-800. Next scan a plastic cuvette and note the region of maximum absorbance. Use the cursor to mark the highest wavelength where significant absorbance occurs.

Using a micropipette, add three mL of 0.5 M sulfuric acid to the cuvette and rescan the blank. Next add approximately 0.5 mL of 1×10^{-3} M Ru(bipy)₃²⁺ to the cuvette with a micropipette. Load and expel the pipette with the solution in the cuvette to mix it well, and scan the absorbance. Use the cursor to locate the maximum absorption wavelength and print the spectrum

Emission Spectra:

Pour the plastic cuvette contents into the ruthenium waste jar and rinse the cuvette with a wash bottle of DIW. The rinsings can go into the sink.

Set the 1000 µL micropipette to 980 and inject three aliquots of 0.5 M sulfuric acid into the cuvette. Using the 100 µL micropipette add 30 µL of 1×10^{-3} M Ru(bipy)₃²⁺ and 30 µL of 0.5 M sulfuric acid solvent to the cuvette. Use the 1000 µL micropipette to mix by filling the pipette and expelling the solution back into the cuvette.

Take an emission scan by exciting at the maximum absorbing wavelength. Use the slit and scan rate settings from Part I, but make the minimum emission scan wavelength 25 nm above the excitation wavelength.

Use the vertical cursor to determine the maximum intensity and wave length.

Verify the excitation wavelength by doing an excitation scan with the emission wavelength set to the maximum value established from the emission scan. Print the excitation spectra indicating the maximum intensity wavelength.

Empty the cuvette into the waste jar for ruthenium and rinse with DIW into the sink. Dry with a cotton swab.

Add another three aliquots of 980 μL of 0.5 M sulfuric acid, 30 μL of 1×10^{-3} M $\text{Ru}(\text{bipy})_3^{2+}$ and 30 μL of the 2×10^{-4} M ferric ion solution. Perform an emission scan and determine the maximum intensity value.

Clean the cuvette as before and repeat the emission scans using of 980 μL of 0.5 M sulfuric acid, 30 μL of 1×10^{-3} M $\text{Ru}(\text{bipy})_3^{2+}$ and 30 μL of the other ferric ion solutions.

Finally, obtain an emission spectra of the 0.5 M sulfuric acid solution to use as a blank.

Record the maximum emission values that correspond with each concentration of ferric ion.

Print out the final plot of the multiple emission scans and indicate the ferric ion concentration that goes with each plot.

Analysis:

Turn in the absorbance, excitation, and emission spectra obtained from the ruthenium solutions.

Present a table with a column for emission intensities (minus the blank intensity for the sulfuric acid solution), concentrations of quenching metals including a zero concentration for the pure ruthenium compound, and a third column for I_0/I values.

Make a Stern-Volmer plot of I_0/I vs $[\text{M}^{n+}]$ (*the ferric ion concentration*).

From the slope of this plot determine k_q/k_s .

Using $k_s = 1.67 \times 10^6 \text{ s}^{-1}$ determine k_q .

Additional consideration:

This section is not required but is presented as an example of how emission spectroscopy can be applied to address questions of kinetics as well as concentration limitations on fluorescence experiments.

The rates of some reactions are controlled by the amount of energy required to overcome an energy barrier such as the electronic repulsion between reacting species as they approach each other. Others are controlled by the rate at which reacting species can diffuse to each other. Determining the quenching reaction rate can help deduce whether the activation energy or the diffusion rate is more important in determining the rate of the reaction.

Activation energy is determined from the equation: $k_q = z_{12} \exp(-\Delta E/RT)$, in which z_{12} is a collision number, and for a solution is approximately $10^{11} \text{ M}^{-1} \text{ s}^{-1}$. For reactions where the rate-determining step is diffusion limited energy is required to bring reactants together against the resistance of solvating molecules forming shells around them. This energy $\Delta E = -10$ to -14 kJ mol^{-1} .

Based on the k_q obtained in this experiment does activation energy or diffusion appear to play a larger role in determining the rate of quenching?

In some cases the signal from a detector will reach a limit and go no higher. This can be the case when stray light is the only light striking and absorption spectrometer detector and the sample has absorbed almost all of the light emitted from the source. It can also occur when no more electrons can flow in a photon or electron multiplier tube such that any increase of light or electron intensity hitting the tube will not be able to generate a larger current. However in these cases an increase in output from the sample causes the signal to stay at a fixed maximum. But if the signal limitation is due to sample itself and not the instrument detector, then the analyst may find the signal, as a function of concentration, will decline after a maximum is reached. Such can be the case when fluorescing molecules begin to absorb emitted light from other excited molecules in solution. As the concentration of fluorophores increases the likelihood of a nearby molecule picking up an emitted photon rises and the number of emitted photons reaching the detector will decline.

References:

- 1. Oxford University 2nd/3rd Year Undergraduate Experiments in Physical Chemistry** <http://physchem.ox.ac.uk/~hmc/tlab/experiments/711.html>
2. Stern, O.; Volmer, M., *Z. Physik.* 1919, 20, 183.

DETERMINATION OF METALS BY PLASMA EMISSION

Reference:

Fassel, V.A. Quantitative Elemental Analysis by Plasma Emission Spectroscopy, Science, 202, 183-191 (1978).

Introduction

The Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) is an instrument designed for simultaneous analysis of several elements. With our instrument up to 25 metals may be determined in as short a time as one minute using about one half of a milliliter of sample. For many elements, plasma emission is more sensitive than flame atomic absorption spectroscopy, but not as sensitive as graphite furnace atomic absorption for the determination of either concentration or absolute quantity. Compared to atomic absorption in general ICPAES is less prone to inter-element interferences, and it will do simultaneous multielement analyses.

The instrument that you will be using is a Jarrel Ash 955 Inductively Coupled Plasma Emission Spectrophotometer. This instrument has fixed channels for 24 elements, a background channel, and an additional channel accessed by a monochromator that can be used to analyze for one additional element selected by the user. On this instrument that channel is often used for potassium analysis.

In this experiment you will analyze 2 elements plus a third used as an internal standard at trace levels in an aqueous sample that simulates an environmental sample such as lake or well water.

You will first use a copper solution to optimize alignment of the refractor plate to direct the maximum amount of light to the photomultiplier tubes. Then you will calibrate the instrument using a blank and a standard, which contains 10 ppm of each element. Multiple standard solutions at the same concentration are needed to avoid the inadvertent precipitation of elements. Deionized water will be used as a blank. After standardization, you will investigate the linear range for one element, copper, observe and attempt to quantify the effect of inter-element interference, and finally determine the levels of the metals in your unknown.

Operation overview

The plasma generated by this instrument emits a tremendous amount of ultraviolet radiation. This is why the plasma is enclosed in a housing with ground glass viewing windows for observation. Avoid looking up at the plasma through the area containing the nebulizer assembly.

Serious eye damage will result from unprotected viewing of the plasma.

The instrument should be fully operational and ready for use when you arrive. The operational commands you will need for this experiment are delineated in the procedure.

You will use two programs to operate the ICP. The first program, called **SLIT** provides a graphic display of the output of up to four of the 26 channels of the instrument. You will use this program to monitor the intensity of light hitting the detector while adjusting the entrance slit micrometer to obtain an optimum alignment between the spectrum and fixed array of exit slits..

The second program is called **C426**. You will use this program to calibrate the instrument and to take data.

The Refractor Plate

A micrometer rotates a 3 mm thick a refractor plate through which light passes after entering the entrance slit. The light then moves directly to the diffraction grating where it is separated into its component wavelengths and directed to the PM detectors. At a micrometer reading of about 650 the refractor plate is normal to the optical path and light passes directly through it as it comes in from the entrance slit just as light held directly vertical to the surface of a glass of water passes directly through it. If the refractor plate is rotated light is refracted through it and redirected to strike the diffraction grating at a different angle causing the light to leave the grating at a different angle and reach the detectors at a different point in space. If the light is diffracted such that it does not reach the detector appropriate for its wavelength with the maximum intensity, the refractor plate can be rotated to change the angle of diffraction and the point at which it reaches the detectors.

Setting the entrance slit micrometer

0. The object of this procedure is to align the entrance slit so that light is directed to a position on the diffraction grating that will give the highest output from the photomultiplier tubes.

1. Start the SLIT program by typing the following commands:

```
C:\> cd progra~1\ICP           moves the user to the ICP directory  
C:\progra~1\ICP > slit        starts the slit program
```

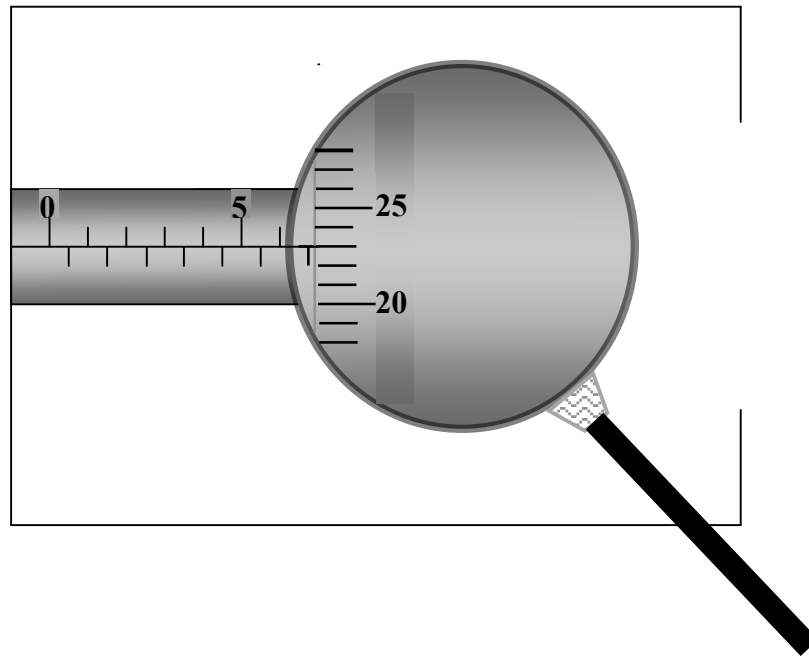
2. Press the **F4** key to select the elements that you will monitor.

3. Select **Cu**. This channel gives a good signal to noise ratio to enable precise alignment of the slit, while also responding in a fashion representative of most of the fixed channel elements. Press the F10 key to close the element selection window.

4. Press **F2** to start monitoring the selected channel.

5. **Measuring Delay Time:** Aspirate the separate 10 ppm Cu solution for the slit adjustment into the plasma. Observe the display and count how long it takes for the readings to increase. This is the time it takes for the sample to reach the plasma. Wait at least this many seconds before starting to collect data during any analyses that follow.

6. Press **F2** to set the reading to 50% on the scale. It will take several seconds for the activated channel(s) to respond.



Entrance Slit Micrometer

Shown through a magnifying glass to enlarge the view.

- 7 Open the cover to the **entrance slit micrometer** and write down the present setting on the micrometer. A replica of the entrance slit micrometer is shown above to help you figure out how it is read. The view is from the top down with the main body of the instrument to the left. For a viewer facing the instrument, up is to the right and down is to the left. A dial numbered from 0 to 50 turns along a shaft with alternating right and left markings. The dial is magnified in the replica below. A full turn of the dial moves the dial the distance from one mark to the next on the opposite side of the centerline. Therefore the distance between alternating markings represents 50 units along the shaft. A zero and a five appear next to two markings on the right of the centerline. These indicate a distance of 500 units along the shaft. To determine the micrometer setting, add the number of units the dial has passed to the number of the mark on the dial that is in line with the center line on the shaft. Notice that the dial on the micrometer below

has passed the 650 mark on the shaft and the 23rd mark on the dial is in line with the centerline of the shaft. So the micrometer setting would be $650 + 23$ or 673.

8. Write down the actual reading you got on the instrument and check with your TA to ensure that you took the reading properly. After your TA approves of the reading you got, adjust the micrometer for maximum output for the selected channel as described below:
 - a) Move the micrometer back and forth from its original setting by rotating the handle. Observe the signal peak on the display as the refractor plate moves through the optimum position. Since the peak is broad, adjust for the optimum position as follows.
 - b) Press the F4 key to rescale the reading to 100%. It will take several seconds for the display to respond.
 - c) There is a line 75% of the distance along the intensity indicator display bar. Turn the micrometer in either one direction or the other so that the display bar reading falls to the 75% line. Note the micrometer reading at this point.
 - d) Turn the micrometer in the opposite direction so that the refractor plate moves through the optimum position and then the display falls to 75% after first passing through the maximum. Note the micrometer reading at this point.
 - e) As the refractor plate is rotated, light is directed across the exit slit leading to the PM (photomultiplier) tube. The width of the beam hitting the exit slit will be wider than the slit itself. When the leading edge of the beam has crossed the slit the signal will increase, rise to a maximum value and change little until the trailing edge of the beam passes across the slit, when it will begin to drop off more rapidly. For this reason the first micrometer reading is taken before the leading edge of the beam has fully crossed the exit slit and the signal is at 75% of the maximum value. Then a second micrometer reading is taken after the most of the beam has crossed the slit and the trailing edge has fallen to 75% of the maximum value. The average micrometer reading between these two positions gives a micrometer setting that is more accurate than one obtained when center of the beam is crossing the slit and little change in intensity of signal is occurring. Record the average of the two micrometer readings taken in steps c and d, and set the micrometer to that value.
8. Hopefully, you have now centered the image of the plasma on the

entrance slit of the spectrograph. If the value is not close to the original setting you wrote down, something is wrong. Ask for help.

9. Press the **F10** key to stop monitoring the selected elements. It may take several seconds for the program to respond.

Setting up for analysis -

Calibration and sample analysis for this class will be done from a program file called: **icp c426**. If it doesn't already exist, a data file: C:\progra~1\icp\chem426.log is created when the **icp c426** program is initiated. Before starting the **icp c426** program type: *del chem426.log* to delete this file from the computer hard drive and remove data from previous users otherwise new data will become appended to old data.

1. To start the program, after the C:\progra~1\ICP> prompt type:
icp c426 *the screen will not have cleared after the slit program terminates. The C:\progra~1\ICP > may be anywhere on the screen.*
2. A screen will appear listing the elements to be analyzed for this lab. ICP analyses often cover 20-30 elements. That number has been reduced considerably for this program in order to be able to accommodate analysis to a three hour lab period. Press **F8** to access the options window. Use the up and down arrow keys to position the pointer and the space bar to select an option. This is not intuitive, since you might expect to use the enter key.
3. Note how many **Bs** are present in the process string. Each B represents a data collection process during which a capacitor is charged by the current emanating from the photomultiplier tube. At present the instrument is set to sum the capacitor readings every ten seconds. In this way the capacitor acts as an integrator to give an average signal that results over the collection period. The standard deviation reading that is displayed during the analysis is the standard deviation for each set of current readings taken from the discharging capacitors.
4. Set the Print Status to On
5. Set the Log Status to On.
6. Set the name to your name or that of your partner.
7. Press **F10** to close the options window.

The RUN LOG below list the order of analysis.

Directions for each step follow the RUN LOG.

READ THOSE DIRECTIONS BEFORE CONTINUING!

RUN LOG

Blank analysis [in the sample mode]while monitoring the intensity readings to verify rinse through of copper after setting the slit.

If blanks are analyzed in the standard mode rather than the sample mode, the calibration data will be wiped out and white space will appear where concentration data should be.

Press F9. Calibrate the instrument in STANDARD mode. [*display will say sample*] Use Blank and Standard 1.

Switch to SAMPLE mode [*display will say standard*].

Analyze the calibration standard as a sample to verify that calibration was successful.

Analyze a blank as a sample to verify that elements used during calibration are rinsed through. Repeat the analysis until the intensity reading of each element does not continue to drop.

Analyze seven blanks to determine the detection limits of the analytes.

Do a 1:100 dilution of the test sample by adding 50 uL of sample to 5 mL of DIW + 50uL of 1000 ppm internal standard.

Do a 1:10 dilution of the test sample by removing 500 uL of DIW from 5 mL of DIW and replacing it with 500 uL of sample. Add 50 uL of 1000 ppm internal standard.

Prepare the straight test sample by adding 50 uL of 1000 ppm internal standard to 5 mL of test sample.

Analyze the above dilutions of the test sample in the sample mode.

Run Standard 1 in the sample mode to determine the amount of instrument drift.

Run the blank to verify that the baseline has not shifted.

Analyze 1000 ppm of Pb (internal standard) to check for inter element interference.

Analyze a blank until all readings return to the baseline.

Analyze 1000 ppm of Cd to check for inter element interference.

Analyze a blank until all readings return to the baseline.

Analyze copper from 0.001 to 10000 ppm.

Allow the system to rinse and verify that the copper readings have returned to baseline before shutting down the instrument.

Calibrating the instrument

0. The Blank: Standards and samples are made by dissolving the elements of interest in deionized water and 1 - 5 % acid. The acid mix may influence the emission intensities in some cases, but for the simple samples being analyzed in this class acid influences will be minimal and deionized water will be used as a blank since it is the major component of the standards and samples.
1. Aspirate deionized water as the blank into the plasma. The terms **F9:Standard** should appear as the second statement from the left at the bottom of the screen. If **F9:Sample** appears instead, press the **F9** key until the word **Standard** appears. Press the **F3** key then the **Enter** key and the instrument will begin taking readings. Note the intensity levels next of the elements displayed. Press the **F3** and **Enter** key again, and, after the screen is updated, notice If the readings change. Copper has been passed through the nebulizer and torch for a long time while setting the refractor plate, so residual copper may be In the system and the copper Intensities may appear to fall for a period of time. Continue to monitor the Intensities by pressing the **F3** and **Enter** keys until the values reach a steady level. They will fluctuate somewhat as an Indication of instrumental noise.
2. Press the **F9** key to move to the standard screen. *Notice that the sample/standard display for the **F9** key on the screen tells you which option you will go to if you press **F9**, not which option you are in.*
3. Verify that the description field says Blank solution. If it doesn't, use the options window (**F8**) to change the Std Solution to Blank. The description field will be automatically updated.
4. Press **F3** to start the analysis of the blank.
5. Aspirate Standard 1.
6. Use the options window to change the Std Solution to 1. Pressing the space bar cycles the Std Solution field through the various standards available. Close the options window.
7. Press **F3** to start the analysis of standard 1.
8. Look carefully at the intensity values for the channels where 10 ppm standards are known to be present. The instrument has been aligned and the gain settings adjusted so that 10 ppm standards will yield counts of 2000 to 80000. Blank values for distilled water should be

low - in the range of 10 to 2000.

Analysis Instructions

The following are general instructions for the analysis of samples in the sample mode, the lab experiment will continue at the section on **Blank Analysis** :

1. If the standard screen is currently active, press **F9** to switch to the sample screen. Remain in this screen for the rest of the experiment unless it becomes necessary to recalibrate the instrument *If analyses are carried out in the standard mode, where the term sample appears beside the **F9**key, the calibration will be wiped out and the instrument will have to be recalibrated.*
2. If the instrument has been calibrated and is still in intensity mode, use the options window to switch to concentration mode using the space bar.
3. Put the sample tube into the solution to be analyzed and wait for the minimum delay time, which was determined earlier when adjusting the slit setting.
4. Press **F3** to initiate the analysis.
5. A window will open to allow you to edit the sample description. Edit the sample description or press the **Enter** key to retain the old description and begin analysis.
6. The analysis will begin when you press the **Enter** key.

Blank Analysis

Verify that the all of the copper used while setting the slit has rinsed through the system by analyzing a blank **in the sample mode [F9: Standard on screen]**, until the copper readings no longer decrease, then analyze the blank sample seven times **in the sample mode** to determine the detection limit of the instrument by multiplying the standard deviation of the seven blank concentration readings by three.

Verification of the calibration

Analyze blank and standards as samples in the **Sample** mode to verify the calibration. The blank should read below three standard deviation units of the seven blanks run in the previous analysis. The standards should all read within 10% of their true value (10 ppm). If they are outside this range the instrument must be recalibrated in the *standard* mode.

Determining the linear dynamic range for copper:

A series of copper standards ranging from 1 PPB (0.001 PPM) to 10000 PPM has been provided, excluding a 10 ppm standard which was used for the calibration and can be reported as 10 ppm. Analyze each of these

standards to determine the upper and lower limit of detection for copper. The lower limit is set by the standard deviation of the seven blank analyses that were done. A reading for a copper concentration below the detection limit usually gives unpredictable results and is hence below the dynamic linear range of the instrument. The upper limit is determined by the accuracy required. If a sample is so concentrated that the atoms entering the plasma begin to absorb each other's radiation, emission intensities will be compromised. For this lab ten per cent accuracy will be required for the unknown that is to be analyzed. If a copper standard reads more or less than 10% of its stated value, then the instrument's linearity has been established only up to the standard that falls immediately below that reading. A test sample that reads above that next lowest standard would be outside the established linear range and must be diluted until its reading is within the linear range.

Internal Standard (IS) :

Rare elements that are not usually found in large quantities such as Yttrium, Scandium, or Indium are used as internal standards for most natural samples analyzed. For the test sample provided however, an element is deliberately omitted so it can serve as an internal standard. If conditions of the sample such as viscosity are different from that of the standards all elements are assumed to be affected the same. By multiplying the observed concentration of an element by the ratio of the expected internal concentration to the observed value the concentration is corrected for the general influences of conditions on the analysis. Internal standard addition will increase the sample volume. This has compensated for when the calibration standards were made, by adding a proportionate volume of internal standard to the calibration standards after the standards were diluted to volume.

The internal standard will be designated and set to a value of 10 during the calibration of the instrument. The following calculation is used to correct for any difference of the internal standard reading from a value of 10 in the test sample.

$$C_{\text{reported}} = C_{\text{analyte}} \cdot 10/C_1$$

Where:

C_1 = Reading of the value of the internal standard in the test sample.

C_{analyte} = The concentration of the analyte read from the test sample.

C_{reported} = The concentration value to report for the analyte in the test sample.

Determination of an unknown:

Distilled water and volumetric glassware are available in the instrument room for your use.

A test sample will be provided to you. Add 50 uL of Internal Standard to 5 mL of the sample. Analyze the sample for the elements in the C 426 program provided for this experiment. If copper is outside the linear range dilute the sample as necessary adding 100 uL to freshly diluted sample that does not already have internal standard added.

Verifying that instrumental drift is within acceptable limits.

Rerun the standards and blanks. The blanks should be below the detection limit and the standards within 10% of their true value otherwise the instrument must be recalibrated and the samples reanalyzed. If there is significant drift and time does not permit recalibration note this in your report.

This analysis has only a two point calibration curve. The uncertainty in

results for this analysis is the difference from the true value of either the first or final 10 ppm calibration standard analyzed in the *sample* mode, which ever is larger.

Observation of inter-element interferences:

Every element emits radiation at multiple wavelengths. One element may emit light near the same wavelength used for analyzing another analyzed at causing an increase in the signal at the second wavelength element of interest. If the interfering element is present in a large amount and the element of interest is present in a small amount, the shoulder of the intensity peak caused by the interfering element will cause an erroneous signal to appear for the element of interest. The interactions of materials in the plasma are complex and sometimes interferences may cause erroneous negative results for the element of interest. After other quantitative analyses have been completed analyze 1000 ppm solutions of the elements provided. Copper has already been analyzed in its linear range analysis and need not be reanalyzed at 1000 ppm. Look for significant (> 3 std of the blank) concentration readings appearing for the other elements displayed besides the element at 1000 ppm. In reporting final concentrations of elements in a test sample use the following calculations to correct for inter-element interferences. Readings for these other elements are referred to as C_{observed} below, because their signal is observed in the solution without the element actually being present. Knowing that 1000 ppm of an element say Arsenic produces a signal for an element such as Cadmium, allows the analysis to calculate a correction factor to subtract off the influence of the interfering element on the element being analyzed. In this case the effect of Arsenic on the Cadmium signal would be subtracted by dividing the cadmium signal in the 1000 ppm arsenic solution by 1000 to find the correction factor, multiplying the arsenic concentration in a test sample by that factor, and subtracting that result from the cadmium reading in the test sample. If more than one element interferes with an element being tested for, all interferences would be subtracted before reporting the final element being tested for. The following equations describe the process.

$$C_{\text{reported}} = C_{\text{readout}} - \sum C_i \cdot F_i$$

Where:

$$F_i = C_{\text{observed}}/1000$$

Where:

C_{reported} = Concentration to be reported of the analyte being interfered with

C_{readout} = Concentration of Analyte in the test sample that was read from the instrument

C_i = Concentration of the Interfering Analyte in the test sample that was read from the instrument

F_i = Factor used to correct for inter-element interference.

C_{observed} = Value of the C_{readout} element that was printed when 1000 ppm of the interfering element was aspirated into the plasma.

Instruments can automatically make the calculations to compensate for internal standard reading variations and inter-element interferences. However in order to become familiar with the way the calculations are done the instrument in this class will not do those calculations. Below is an example of how to correct for changes in internal standard readings and inter-element interferences.

A sample was analyzed that contained silver and also arsenic and iron with an internal standard of 10 ppm lead.

1000 ppm of Arsenic (As) gave a Silver (Ag) signal of 5.762 ppm.

$$F_{\text{As}} = 5.762/1000 = 0.005762.$$

1000 ppm of Iron (Fe) gave a Silver (Ag) signal of 0.985 ppm

$$F_{\text{Fe}} = 0.985/1000 = 0.000985.$$

The sample gave the following readings:

Read out values:

Ag: 1.238

As: 35.487

Fe: 157.621

Pb: 8.752

$$C_{\text{reported}} = (C_{\text{read}} - \sum C_i \cdot F_i) \cdot 10/C_I$$

$$\text{Ag}_{\text{Report}} = [1.238 - (0.005762 \cdot 35.487 + 0.000985 \cdot 157.627)] \cdot 10/8.752.$$

$$\text{Ag}_{\text{Report}} = 0.984 \text{ ppm.}$$

Data retrieval:

Data is stored in a chem426.log file. Take a copy this file with you from the C:\progra~1\icp directory.

The .log is a text file that can be printed out from note pad or MS Word, however the data in the file is not as straight forward as the data shown in the printout. Therefore the data from the printer should be used and the .log file kept for a backup.

Pre-Lab Questions (10 pts.)

1. What is the "blank" for this instrument and why is it used (two sentences maximum)?
2. What two programs will you use to optimize the ICP operation and to collect data (one sentence maximum)?
3. What is the purpose of the entrance slit micrometer? Why are two micrometer positions determined (two sentences each maximum)?
4. How will you investigate inter-element interference?
5. What is the function of an internal standard?

Data Analysis (30 pts.)

6. Make a table of the elements you analyzed **not** including the internal standard, and their detection limits from your data. Also include in this table the uncertainties (+/- 1 sigma) printed out for each element of the 10 ppm standard solutions.

Explain in a note following the table how you determined the detection limits (using the seven blank runs) and show a sample calculation.

7. Make a table of the elements that are interfered with by other elements. List the interfering element and the factor by which that elements concentration must be multiplied to correct for its interference (F_i).
8. List the concentrations of the metals in the test sample corrected for internal standard variation and inter-element interferences. The complete formula for the test sample concentration calculation is:

$$C_{\text{reported}} = (C_{\text{read}} - \sum C_i \cdot F_i) \cdot 10/C_I$$

Give the standard deviation between the two ten second analyses for each element analyzed. Finally, include the uncertainty based on the largest deviation either the beginning or ending calibration standards and their expected value.

Post-Lab Questions (10 pts.)

9. How is the inductively coupled plasma formed and sustained?
10. Name two causes of nonlinearity in calibration plots over a large range of concentrations?
11. What is a the major cause of inter-element interference?
12. What can be done to correct for inter-element interference and produce more accurate data.
13. A chemist analyzed a sample high in arsenic on an ICP-AES and was surprised that cadmium showed up. She became suspicious and ran a standard of pure arsenic. The cadmium showed up there too. Could the arsenic standard be contaminated with cadmium? She decided to analyze the arsenic standard with an atomic absorption spectrometer. What should she analyze for and how could that analysis determine if the standard was contaminated?

revised 2/09

Appendix A

INFORMATION FOR TAs

Starting and Shutting Down the Inductively Coupled Plasma Spectrometer

Initiating the computer:

1. Switch on the power bus strip on the floor behind the computer.
2. Verify that the computer, monitor and printer have been turned on.
3. Change to the ICP directory on the computer.

```
C:\ >cd\progra~1\icp
```

Igniting the plasma:

1. Visually check the alignment of the torch. Check that the torch is centered inside the load coil. The plasma will not ignite if the torch is not aligned properly. Do not attempt adjustment of the torch alignment if you are not familiar with the procedure.
2. Make sure that both exhaust fans are operating.
3. Flip the **line** and **control** switches on the RF generator upward. These are located on the lower right on the front of the RF generator.
4. Push the **alarm reset** button on the RF generator to deactivate the alarm that sounds when the RF generator is first turned on.
5. Open the main valve on the argon tank (the one leading to the regulator). Set the outlet pressure on the regulator to between 30 and 40 psi. Note that since there is liquid argon in the tank rather than compressed gas, the primary gauge reads the vapor pressure of liquid argon, which can range from 30 to over 200 psi. There is a pressure buildup valve on the other side of the tank that can be opened for a short period of time if the proper flow rates cannot be achieved. Be sure not to leave the pressure build-up valve open.

To determine whether the tank is empty **a)** observe the gauge on the top of the tank or **b)** try to tip the tank - it will tilt easily if it is

nearly empty.

6. Slowly open the **coolant** and **plasma** gas toggle switches located on the front of the plasma housing.
7. Adjust the needle valves (bottom of the beads) to:
coolant gas 19 liters/minute (open all the way)
sample gas maximum flow (0.75 SCFM = 0.4 liters/minute)
plasma gas 1.0 liter/minute
8. Check the sink to make sure that the coolant water is flowing. Only a very small flow is required.
9. Tension the pump tubing. Aspirate deionized water at 1 ml/minute for at least three minutes. Make sure that the nebulizer connection is not leaking. Check that a mist is forming in the nebulizer chamber.
10. Set the controls on the RF generator:
power control manual
local switch local
incident control dial as indicated
lower horizontal switches on, on, off (left to right)
11. Set **power** dial fully counterclockwise.
12. Set **capacitor** on side of plasma housing to the value indicated.
13. Turn off the peristaltic pump and the sample gas flow.
14. Close the front door of plasma chamber. The blue **RF off** button on the RF generator will light when the RF generator has warmed up.

CAUTION: If anything appears wrong while lighting the plasma, quickly open the front door of the plasma chamber. This will extinguish the plasma.

15. Check igniter discharge by pressing the **igniter** button and adjusting the knob on the bottom of the tesla coil (underneath the plasma housing) until the arc is confined within the torch.
16. Push the red **RF on** button. The plasma will not light if the red button is not lit.

17. While pressing the **igniter** button (hearing discharge), rotate the **power** dial clockwise until the plasma is visible in the viewing window and the incident power is about 1 KW. The plasma will light more easily if the dial is turned fairly quickly.
18. Make sure that there is no red glow or yellow tail visible through the viewing window --if there is, quickly open the front door of the plasma chamber and try igniting again.
19. Rotate the **capacitor** dial (on the side of the plasma housing) to minimize the **reflected RF power** meter on the RF generator. If you can not obtain a value below 30 watts the torch may be out of alignment.
20. Flip the **power control** switch to **auto**.
21. Minimize the reflected RF power (watts) by again rotating the **capacitor** dial.
22. Rotate the **power** dial fully clockwise. A discharge may be heard.
23. Minimize reflected RF power.
24. With the **incident control** dial, adjust the **incident power** meter to the intended power. 1.0 KW is the normal operating power.
25. Minimize reflected RF power.
26. Observe plasma through the viewing window. Make sure there is no red glow coming from the top of the torch indicating the torch is overheating, or yellow tail in the plasma below the top of the torch indicating a leak in the lower torch fittings.
27. Very slowly turn on the **sample** gas. Watch the bottom of the plasma rise inside the load coil. If the bottom of the plasma reaches above the second coil the plasma usually extinguishes. Keep the plasma below the second coil by reducing the **plasma** gas flow in small increments while increasing the sample gas flow to its maximum. Be careful not to let the plasma get too close to the top of the torch. A darker core should appear in the plasma as the sample gas from the nebulizer cools the center of the plasma.
28. Reduce the plasma flow if necessary so that the bottom of the plasma is visible below the first coil. It should not be necessary to

reduce the plasma flow below 0.6 liter/minute.

29. Turn on the peristaltic pump and aspirate deionized water.
30. Minimize reflected RF power.
31. Allow the plasma to stabilize for 15 to 30 minutes before analysis.
It is not a good idea to leave the lit plasma unattended.

Optimizing the plasma imaging mirror:

Vertical and horizontal alignment of the plasma imaging mirror is adjusted using the two micrometers behind the access door on the right side of the plasma housing toward the front. The second door toward the rear of the instrument allows access for adjusting the imaging mirror for the N+1 (monochromator) channel. Only the vertical alignment needs to be adjusted to optimize the signal to noise ratio for a given element or group of elements. The vertical alignment micrometer is the upper knob. **Be careful not to look at the plasma while adjusting the imaging mirror micrometer.**

The black marks on the rectangular window on the front of the plasma housing are used as a guide to the position of the plasma image. Adjustment of the imaging mirror so that the lowest of the three marks coincides with the top coil has been found to give optimum signal to noise ratios for most of the elements.

To adjust the image for a particular element, measure the intensity counts of a standard containing that element, measure the intensity counts for deionized water, and then calculate the ratio of the standard to background intensities. Slightly change the vertical position of the imaging mirror, measure the standard and blank again, and calculate a new ratio. Repeat this procedure until the optimum vertical alignment of the imaging mirror is found.

Shutting down the instrument:

1. Extinguish the plasma by opening the front door of the plasma chamber.
2. Aspirate deionized water for at least three minutes.
3. Return all switches on the RF generator to the original settings.
4. Reset the capacitor dial to the indicated value.
5. Turn off the main valve on the argon tank.
6. Lower the toggle valves for the coolant and plasma gas flows after all the argon has bled from the lines.
7. Turn off the peristaltic pump and release the tension on the pump tubing.
8. Replace the dust cover on the monochromator.
9. Exit the program by pressing **F10** twice.
10. Park the heads on the hard disk:

C:\ICP > LO

11. Turn off the computer
12. Turn off the power bus strip on the floor underneath the computer.
13. Leave the capillary in deionized water.
14. Sign the log sheet.

FLOW INJECTION ANALYSIS

The purpose of this lab is to illustrate some of the principles of flow injection analysis (FIA) and to demonstrate a simple application. A FIA system has been configured for the determination of dissolved aluminum in freshwaters. First, the dispersion of this system will be characterized and then the system will be calibrated and some unknown samples analyzed. Finally, the reproducibility, linear dynamic range, and maximum sample throughput will be determined.

Introduction

An excellent book describing the fundamentals and applications of FIA has been written by Professor J. Ruzicka of this university and Dr. E. Hansen of Denmark (1), two of the founding fathers of FIA. Students are advised to refer to this book for a more in-depth discussion of FIA than follows here.

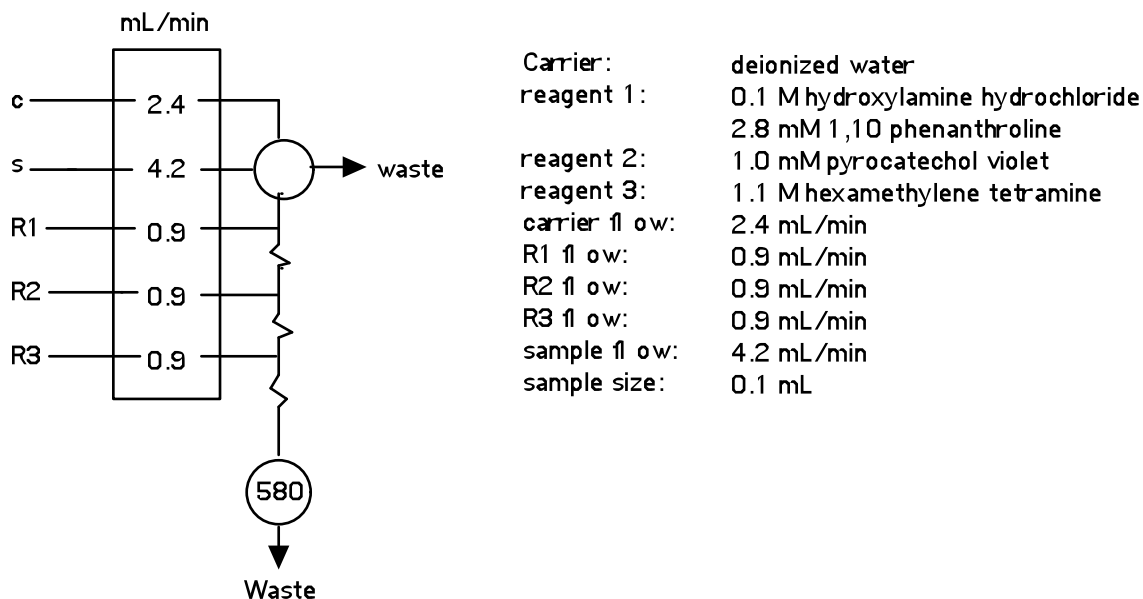
Since the publication of the first papers describing FIA, hundreds of applications for its use in environmental, industrial and clinical analyses have been reported. The adaptation of batch or segmented-flow colorimetric methods to FIA is a common approach. Here, a reproducible sample volume is injected into a flowing carrier stream, and subsequently (i.e. downstream) reagents are added to the flow path at tees and the stream is delivered to a flow cell where detection of the analyte occurs. Detection is typically performed by measuring the absorbance of an unknown sample and comparing it to a calibration curve obtained by the analysis of standard solutions. The advantages of FIA include reproducible volumes and mixing, reduced sample handling, and fast analyses.

Background

The method used in this laboratory is a simplified version of the method for determining dissolved aluminum species in freshwaters presented in reference 2. The impetus for developing the method was the toxicity of dissolved inorganic aluminum to fish seen in lakes in the Northeastern U.S. and Scandinavia as a result of acidic deposition ("acid rain"). The U.S. EPA desired a sensitive method to determine the concentration of dissolved aluminum species in samples without affecting the carbonate equilibrium of the sample, which has been shown to affect the speciation of dissolved aluminum. This demand precluded the use of previous segmented-flow methods where an air bubble directly contacts the

sample, and required the collection of samples in air-tight syringes. Thus it was decided to adapt existing chemistry to an FIA method with these constraints in mind.

This method utilizes the complexation of aluminum with pyrocatechol violet (PCV), a strongly absorbing dye. The PCV also complexes iron (III) which can cause an interference if not considered. Therefore it is necessary to reduce iron (III) to iron (II) with hydroxylamine hydrochloride. The reduced iron is then complexed with 1,10-phenanthroline thereby effectively masking any iron present in the samples. The PCV is added subsequent to the iron masking so that only aluminum is complexed. The pH of the sample is then adjusted to approximately 6.2 with hexamethylenetetraamine (HMTA), where the best signal-to-noise ratio is obtained. This pH is also close to the pH of most samples, thereby minimizing the change in pH of a sample. The absorbance of the aluminum-PCV complex is then measured in a flow cell at 580 nm. A schematic diagram of the method is included as Fig. 1. The carrier stream is deionized water and the injected sample volume is 0.1 ml.



Operating instructions for Computer Controlled FIALab 2500 Flow Injection Analysis

Double click on the *FIALab for Windows* icon.

Click on the *Instruments* header, then *systems* and observe if the *FIAlab-2500* instrument is checked. If it is not, click on it. Be careful not to deselect this instrument by highlighting it if it is already checked.

Setting Up the Spectrometer

The spectrometer collects light from the lamp source through a fiber optic cable. Light is passed through a flow cell through which a sample passes before reaching the spectrometer detector. The various wavelengths of from the light source strike a diffraction grating and are dispersed across a photo diode array strip. This is a strip of photodiodes from which a voltage is measured from each diode as light strikes it. Since a different narrow band of wavelengths is striking each diode, the voltage channel used measure each diode is a measure of the intensity of light at specific wavelengths. The samples in this case will absorb light across a fairly broad band of wavelengths. Hence several channels can be used simultaneously to measure absorbance. The more channels used the larger the voltage signal read. The sample will also take several seconds to pass through the flow cell allowing several voltages readings to be taken in sequence and averaged to increase the signal to noise ratio and hence the sensitivity of the measurements. The spectrometer can also respond rapidly enough so that each reading in the sequence can be measured or integrated over several milliseconds allowing the voltage signal to increase during each measurement period and hence help increase the magnitude of the signal.

Click on the *Spectrometer* button

Go to the *Spectrometer Graphing Window*

Click on *Login*

The top *Master* wavelength window should contain a value between 578 and 582.

If the wavelength in that window is outside those values, click on the button that shows the wavelength value.

With the mouse, move the cursor into the Voltage/Wavelength (top) window so that the cursor is above the point on the Wavelength axis at a value between 578 and 582.

Click the mouse and note the reading on the top *Master* wavelength button. If it changes to a value outside 578 and 582, reposition the cursor and click it again until a value within that range registers on the button.

Click again on the top button showing the *Master* wavelength to permanently set the wavelength being recorded.

Disabled should appear in the windows of the second, third buttons.

A value between 385 and 415 should appear in the window of the fourth button and it too should read *Master*. If it does not change it to read *Master*. Change the wavelength if necessary as was done on the first button.

Click on the *Spectrometer Set Up* tab

You should see the following parameters:

Integration Time:	in msec will be set on a case by case basis.	
Detectors to Average:	will be set on a case by case basis.	
Samples to Average:	will be set on a case by case basis.	
Sample Rate:	2 - 4 Hz	
First Coefficient:	0.3671855	<i>These parameters may vary from spectrometer to spectrometer and are set by the manufacturer, so don't be concerned if they differ on this one..</i>
Second Coefficient:	-1.49761E-05	
Third Coefficient:	-1.86982e-09	
Intercept:	189.1604	
Hardware:	OOD Driver	
Selected Spectrometer Master :	Enabled	
Use Wavelength 4 as reference		
For Master Spec.:	√	

Correct any values that need to be changed.

The light source is a tungsten lamp connected to the sample cell.

If the lamp is on, turn it off with the switch in the back.

Click the *Spectrometer Graphing* tab to go to the *Spectrometer Graphing* window.

Verify that the *Repeat Scan* button is **not** highlighted. If it is, click on it.

Click the *Dark* button on the bottom of the screen. Above 200 nm a flat line with possibly two or three small, broad beaks should appear at near 150 volts.

Turn on the lamp.

Fill the syringe leading to the sample cell with DIW and inject it into the sample cell.

Click on the *Reference* button.

The voltage/wavelength curve should not have any sharp corners to it above 200 nm.

If it does, then the spectrometer is too sensitive to the light entering it. Ask the TA for assistance in reducing the spectrometer sensitivity. The maximum reference voltage should be at least ten times the dark voltage. It is important not to click on the *Reference* button with a sample in the sample cell or the spectrometer will calculate all subsequent absorbances based on a zero absorbance value for the sample cell containing sample. If the *Reference* button gets accidentally clicked on, then a new reference scan must be done with only the carrier solution in the sample cell.

Click on the *Absorbance* button.

Determining the Dispersion Coefficient (D)

The dispersion coefficient for a particular configuration of a FIA system is the ratio of the concentration of a undispersed material, C_0 divided by the concentration of that substance, C , after it has been dispersed from the sample loop and arrived at the detector. That is:

$$D = C_0/C$$

Fill the syringe with blue dye (food coloring).

Click on *Repeat Scans*.

Slowly inject the blue dye into the sample cell.

The absorbance time plot should climb to a plateau and level off before you have completely injected the entire sample. If the injection is complete before a plateau has been reached then the blue dye has not completely rinsed out the DIW that was previously in the cell and a second syringe of blue dye should be injected. Leave the syringe in place after injecting the dye.

After an absorbance plateau is reached let the spectrometer continue scanning for 10 – 15 seconds, then click on *Repeat Scans* again to stop the scanning.

The absorbance reading along the top of the plateau is proportional to the C^0 value required to calculate the dispersion coefficient, D . This absorbance value can be estimated by using the mouse to place the cursor on the voltage/time screen along the flat part of the top of the curve, clicking the left mouse button, and reading the Mouse Y position on the left side of the plot.

The *Data Analysis* option offers more complete information.

Return to FIALab for Windows screen and select *Analysis*.

Adjust the window that appears so that the buttons on the bottom can be seen.

Click on the *Plots* tab if the plots screen is not already visible. If any plots appear on this screen they are the result of data obtained prior to the most recent analysis.

Click on *Refresh*. Current data will be displayed as a graph on the Time Series Channel representing an absorbance/time plot.

Find the time where the values have reached a plateau.

Click on the *Data* tab. An Excel spreadsheet will be displayed containing the time and absorbance data recorded from the most recent scan.

Channel 1 is the only channel to collect data. Look down the Time column until the values are those where the curve reached a plateau on the plot screen.

The absorbance values at that point can be read in the Value Column. This column may need to be widened to see all of the decimal values in the column.

Several absorbances may be manually recorded and averaged to obtain a value proportional to C_0 . Alternatively the data can be saved as an Excel file by clicking on the *Save* button at the bottom of the *Plots* screen. From Excel the Average formula for several cells in the value column can be used to calculate the average value.

The following instructions are for setting up the pump, computer program and will be used to determine an absorbance value proportional to the C component of the dispersion coefficient, D .

Unscrew the syringe injection port from the sample cell and connect the exit tube from the small Teflon reaction tower to the sample cell.

Click on the *FIALab* button from the *FIALab for Windows* screen.

Click on *Login*.

Set the pump speed to 50%.

If the injection valve diagram does not indicate that the valve is in the load position, click on it so it says LOAD.

With the TA's help place the pump tubes for the sample and carrier stream in the FIA pump and snap the clamps in place.

Click on the pump symbol to turn on the FIA pump.

Place all of the pump tubing in a water reservoir.

With the TA's help place the pump tubing in the *ISMATEC* pump, set the pump speed to 20, turn on the pump, verify that the pump rotor is turning in a direction that will deliver solution to the reaction tower, and ratchet the clamps to tighten them one tube at a time until water just starts to flow in the tubing.

Return to the *FIALab for Windows* option and select *Programs*. Position the window to see the buttons on the bottom. Open the file:

System C:\program files\WINFIA5.0\Chem426.fia.

This program contains all of the steps used in the rest of the analyses for this lab session. Examine the steps in the program and try to understand the sequence of events that will be executed as the program proceeds.

Place the sample tube in the blue dye and all other tubes in DIW.

Click on *Run* to start the program and notice that the program steps are highlighted in black as they are executed. You may go to the *Spectrometer Graphing* screen or *FIALab-2500* screen to observe the absorbance plot as it is acquired.

As the dye (or AI during the analyte analysis part of the experiment) is flushed from the sample loop by the carrier, it goes through the reaction tower and is mixed and diluted each step of the way by the liquids flowing into the reaction vessel. The plug of sample flows into the sample cell. The sample shows up as an absorbance peak called a FIAGRAM. These peaks have a characteristic shape based on the way the sample is diluted along the length of the tubing it is carried through.

When the program finishes, the maximum peak absorbance value may be estimated using the mouse on the Absorbance/Time plot from the *Spectrometer Graphing* window or by

refreshing the *Plots* screen in the *Analysis* option, going to the *Data* window and finding the maximum value in the Value column of Channel 1.

When running the blue dye the maximum for the absorbance peak is proportional to the concentration value for C in the dispersion coefficient equation.

ANALYTE ANALYSIS

Place the reagent tubes from the *ISMATEC* pump into their appropriately numbered reagent bottle and allow enough time for all of the reagents to flow through the entire system.

Analyze each of the standards by placing the sample tube in the bottle of the standard to be analyzed and running the program. No peak will be seen at the lowest standard if it is below the detection limit. Higher standards may not have peaks proportionally higher than lower standards because they are beyond the linear range of the spectrometer.

Pick a low standard just above the detection limit and analyze it seven times and pick a standard in the mid range of the standards and analyze it seven times also in order to compare deviations in the data.

Mix equal volumes of two standards for a test sample and analyze it.

Shutting down

When finished run water through all of the tubes for at least five minutes. Then release the tension on all of the pump tubing and release one end only of each tube from its fastener.

Reconnect the syringe and syringe port to the sample cell.

Shut off the lamp.

Turn off the *ISMATEC* pump.

End the FIA program.

FIA Data Analysis

The Excel file with the FIA data contains extraneous information. The last row of the data is usually extraneous and should be deleted in Excel. Only the first two columns that begin with actual time and absorbance data should be imported into Matlab. All of the rest of the data must be deleted for Matlab to accept just the useful data. Data can be loaded into Matlab using the import data option in the file menu. A

variable with the same name as the data file will then appear in the Matlab workspace.

There is a matlab program called `peakcalc` with the `chem426.m` files. This file may have been revised since you collected your `.m` files at the beginning of the quarter so be sure you have the most recent version before leaving the lab. The file is invoked by typing `peakcalc` and entering, at the prompt, a *variable name* to store the peak time, height, and area values. The `peakcalc` program contains within it other programs that are not activated unless the mouse button is clicked. For those programs to know the variable name it also has to be entered once the program is running, hence the user is prompted to reenter the variable name. After entering the name for the variable and pressing the ENTER key, a plot of the absorbance vs time will appear on figure 1.

Run the `peakcalc` program for each analysis that was performed and save the values in the variables you name. While viewing the figure created use the mouse to move the cross hairs over the shoulder of the peak a fixed distance from the peak maximum and click the left button. This will give the value of the peak at that time position which is Information that will be needed to do part 8b In the Data Analysis below. The data variable you named when invoking the `peakcalc` program will be updated each time you click the mouse button, and you can see this by typing the variable name from the Matlab command line.

Make plots of peak area vs concentration and peak height vs concentration. Use the `datplt` program to plot out the data within the linear range and determine the concentration of the test sample.

Pre-Lab Questions (10 pts):

1. Define dispersion as it relates to Flow-Injection Analysis. What does a value of $D=2$ mean in terms of sample dilution?
2. What will prevent iron from interfering with the aluminum measurements?

Data Analysis (30 pts)

4. Calculate the dispersion coefficient (D) for the FIA system. Show your calculations and include all data.
5. Plot the FIA-grams (Absorbance vs time) of the standards so they overlay each other. The hold on plot option can be used for this.
6. Make two plots. One of peak area vs standard concentration and another of peak height vs standard concentration.

To determine the correlation coefficient of the calibration lines type: Use Matlab's `corrcoef(x,y)` function, where x is the concentration vector and y is either the peak area vector or the peak height vector. Report this correlation coefficient (r^2) for both sets of calibration data. Note: the `corrcoef` function will give a matrix output of correlations of

x with x	x with y
y with x	y with y

Since x will always correlate perfectly with x, the upper left value will always be 1. Since y will always correlate perfectly with y, the lower right value will always be 1. The upper right and lower left values will be equal to the correlation coefficient value sought.

7. Determine the standard deviation of the peak areas of the one and ten ppm standards. Determine the standard deviation of the peak heights of the one and ten ppm standards. Report the mean, standard deviation, and % relative standard deviation of the peak height and peak area values.
8. Based on how do the SD's and %RSD's of the peak area vs the peak height compare, which measurement should give the best reproducibility?

Post-Lab Questions (10 pts)

11. If sample absorbance readings went above the linear values of the spectrometer, give two examples of how the FIA system that could be adjusted to increase the value for D, hence causing the sample to disperse more and pass through the flow cell at a more diluted concentration. (Assume that if the system is altered by these suggestions the standards would also be reanalyzed under the new configuration.)
12. If the absorbance readings went above the linear values of the spectrometer but the value for D had to remain fixed, describe how another part of the system or instrument could be altered to decrease the sensitivity and allow the readings to remain within the linear range of the spectrometer.

References

1. Ruzicka, J. and Hansen, E. Flow Injection Analysis, 2nd ed.
2. Henshaw, J.M., Lewis, T.E., and Heithmar, E.M. Intern. J. Environ. Anal. Chem. (1988), Vol. 34, pp. 119-135.