

## Spectroscopic Instrumentation Lab

### *Molecular Absorption (UV/Vis) Spectroscopy*

#### I. BACKGROUND

##### A. Theoretical Background

Absorbance spectroscopy is a widely used analytical technique. At its very core, this method involves comparing the light transmitted through a blank to the light transmitted through an absorbing species. If the incident light (at a specific wavelength,  $\lambda$ ) has intensity  $I_0$ , and it passes through a sample that absorbs at the wavelength  $\lambda$ , the light intensity,  $I$ , leaving the sample is attenuated. These two quantities are related by the molar extinction coefficient at the wavelength of interest,  $\epsilon_\lambda$ , the concentration of the absorbing species,  $C$ , and the length of the path that the light traverses through the sample,  $l$ .

$$I = I_0 e^{-\epsilon_\lambda C l} \quad (1)$$

This is a form of Beer's Law, and by manipulating the equation, the following form can be achieved.

$$-\log \frac{I}{I_0} = \epsilon_\lambda C l \quad (2)$$

The quantity that is measured in absorption spectroscopy is the logarithmic term, which is commonly called the absorbance. By substituting in  $A$  for the  $-\log$  term, we arrive at the common form of Beer's Law.

$$A = \epsilon_\lambda C l \quad (3)$$

Absorbance is a ratiometric measurement, which requires you to measure the ratio of the intensity of light transmitted through your sample to the intensity of light transmitted through a "blank". (**The blank should be identical to the sample in every way except that the molecule of interest should be present at a concentration of zero.**) With a dual beam instrument this ratio is measured directly, and the user installs both a blank and a sample into the instrument. Diode array instruments are single beam instruments, and the blank and sample must be measured sequentially. **The blank is always measured first!!** With the HP 8453, when the blank is measured it is stored in a register which identifies it as the blank. The most recently measured blank is always used to calculate the absorbance spectrum of all subsequently measured samples.

*Note: If the conditions of the solvent system change you must measure another blank before measuring the sample. Also, if a long period of time has elapsed after the last blank measurement, it is wise to measure another blank just before measuring the sample. This small investment in time will improve the accuracy of the measurement by minimizing the effects of long-term light source drift on the spectrum.*

## B. Instrumental Background

The Hewlett Packard 8453 is a photodiode array UV/Vis spectrometer. This type of instrument allows for very quick measurements of absorption spectra of a large wavelength range. This is accomplished by the fact that it is NOT a scanning spectrometer. Rather, it detects all wavelengths simultaneously, by utilizing an array of photodiodes on a single grid, each detecting a specific wavelength. The light passes through the sample from the lamp, and then is dispersed and projected onto the photodiode array. The light is dispersed in such a way that specific wavelengths fall on VERY specific areas of the diode array.

The obvious advantage to the 8453 is the short amount of time required for a measurement. With scanning models, which scan the excitation source through the UV/Vis spectrum by use of a scanning monochromator, measurements may take more than 5 minutes. However, by making use of the ability to simultaneously detect all wavelengths at once, the HP 8453 can make a measurement in about 5 seconds. The major disadvantage to the diode array instrument is the limited resolution. Scanning instruments, depending on the resolution of the excitation monochromator, are able to achieve resolutions easily on the Angstrom scale (0.1 nm). The HP 8453 has 1 nm resolution, and its resolution cannot be changed.

Another distinct advantage of the HP 8453 is its high degree of user-friendliness. The software is relatively easy to use, while also being rather powerful. On top of making absorption measurements, derivative spectroscopy is also an option, which is quite useful in the measurement of samples with high backgrounds. The software is also able to determine peaks in the spectrum and list their wavelengths as well as their absorbance at that wavelength.

## II. Instrument Setup

- A. From the INSTRUMENT menu choose spectrophotometer SETUP, and set the wavelength range of interest. Note that a narrower range will not improve the resolution, but it will present an expanded display of the data which may be easier to read. The full range of the instrument is 190–1100 nm, although the most practical range for measurement falls within 200–800 nm. For these measurements, we will measure the entire spectrum from 190–1100 nm, and zoom in on areas of interest after the measurement has been taken.
- B. From the instrument menu, choose LAMPS. A filled circle inside the open circles to the left of the lamp types indicates that the lamps are on. Click the mouse on the circle to turn lamps on or off. Make sure both lamps are on before collecting spectra.
- C. From the METHODS menu, choose SETUP ANALYSIS.
  1. Set the desired wavelength range in the INSTRUMENT SETUP dialog box.
  2. Make sure that the DISPLAY SPECTRUM range in this dialog box matches the wavelength range in the INSTRUMENT SETUP dialog box.
  3. Choose the ANALYSIS MODE: absorbance, first derivative or second derivative. Note that this item can be changed after the spectrum has been recorded, and both the spectrum and the peak results will be given for this spectrum.

- D. From the METHODS menu, choose SPECTRUM/PEAKS. Click on the circle on the left to tell the software to find and annotate peaks and valleys. This can also be done after the spectrum has been collected.

### III. Making Measurements

#### Cell Care

Spectrophotometric cells must be handled properly if reliable results are to be obtained. A few helpful rules are listed:

1. Inspect for scratches on the windows. Never touch the optical surfaces of cells with your fingers.
2. Either use a single cell for an entire series of measurements, or assure that all cells used are transmittance matched for path length.
3. When filling cells, rinse them thoroughly with the solution to be measured, then fill, assuring that no air bubbles adhere to the windows. Wipe the outside of the cell with ethanol and a Kimwipe. *Never use a paper towel.*
4. Place cells into the instrumental carefully, assuring that they are properly seated in the holder. Use the same cell orientation each time.
5. If a volatile solvent is being used, place a cover on the cell to reduce evaporation.
6. Never store solutions in cells. Rinse thoroughly when finished and allow to dry in a dust-free environment. Basic solutions are especially likely to etch cells if they are not properly cleaned.
7. Always keep cell in the cuvette holder when they are not in an instrument.

#### General Procedure for using the HP UV/Vis Photodiode Array Spectrometer

**\*\*\* THE INSTRUMENT AND LAMP MUST WARM UP BEFORE USE! \*\*\***

#### Procedure for making a measurement

- A. Install the blank solution in the cell holder. Choose the MEASURE menu and click the left mouse button on the BLANK menu item. The software will indicate that it is busy in the lower right corner until the measurement is made, and then it will display the blank spectrum. You are now ready to measure your sample. **Remember that the blank must be identical to the sample of interest in EVERY WAY with the exception that the analyte must have a concentration of 0.**

- B. Place the sample solution in the cell holder. Choose the MEASURE menu, and click on the SAMPLE menu item. In about 15 seconds the spectrum will appear on the upper portion of the monitor screen. In the lower portion is a RESULTS window. This will contain a listing of peak wavelengths and absorbances. If no data is present in the RESULTS window, go to the METHOD menu, SPECTRUM/PEAKS item and enable **Find and Annotate Peaks**. Make sure that the number of peaks to be annotated is high enough to list all peaks of interest.

#### IV. Printing and Saving

- A. To save a spectrum, go to the FILE menu, and choose SAVE. Give the program a filename. The file type must be SD.
- B. To print your results you will need to save your data to a floppy disk as a .WAV file. You can then transfer the file to the computer in room 102. Start EXCEL and import the data. You will then need to make a plot of your spectrum, which can then be printed.

#### V. Samples to Run

Four samples will be run in this lab, after the wavelengths have been calibrated. To make sure that the wavelengths are properly calibrated, a Holmium Block is provided with the UV/Vis instrument. Holmium has very distinct absorption lines, and therefore, it is a perfect sample to use to make sure that the wavelengths are calibrated. To measure the absorbance of the Holmium Block, an air blank must be collected first, which means you must take any sample out of the sample holder and simply collect a BLANK spectrum with no sample present. Then, simply place the Holmium Block into the sample holder (such that the transparent part is in the window of the sample holder), and measure the spectrum. The spectrum should exhibit peaks at the following wavelengths: 287.0, 361.1, 450.8, 537.0, and 640.4 nm.

The rest of the samples will require solvent blanks, rather than air blanks. The rest of the samples are as follows:

1.  $1.2 \times 10^{-5}$  M Perylene in Hexane
2.  $1.2 \times 10^{-5}$  M Perylene in Ethanol
3.  $1.0 \times 10^{-5}$  M Dimethylaminobenzonitrile (DMABN) in Hexane
4.  $1.0 \times 10^{-5}$  M DMABN in Ethanol

Remember that when the solvent is changed, another blank must be taken. Therefore, when measuring the perylene spectra, first a hexane blank should be taken with the pure hexane provided, then the perylene sample in hexane should be taken. After which, an ethanol blank should be taken with the pure ethanol provided, and then the perylene in ethanol spectrum should be taken.

Print out the results, along with the numerical results, and use the results and the concentrations given above (along with the knowledge that the path length of the cell is 1.0 cm) to **calculate the molar extinction coefficients of each of the peaks in the spectra for perylene and DMABN in the different solvents.**

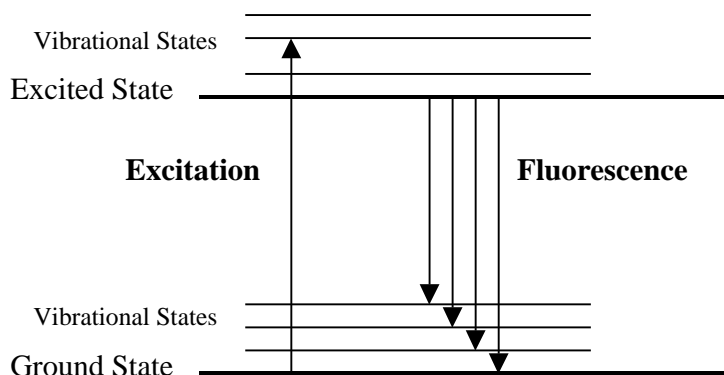
## *Molecular Fluorescence Spectroscopy*

### I. Background

#### A. Theoretical Background

Fluorescence spectroscopy is a form of analysis that utilizes the emission properties of specific molecules rather than their tendency to absorb certain wavelengths of light. This very fact makes it inherently more selective than absorption spectroscopy, due to the fact that all molecules that fluoresce must absorb, but not all molecules that absorb necessarily fluoresce. Therefore, only specific types of molecules can be studied using fluorescence spectroscopy.

First of all, it must be recognized that absorbance and emission (fluorescence) spectroscopy are closely linked. In order to have fluorescence, a molecule must be in an excited state, and in most cases this is achieved through the absorption of a photon that is equal to the energy gap between the ground state of the molecule and some excited state. Once the molecule is excited, a number of relaxation pathways are possible that allow the excited molecule to release its excess energy. In many cases, the relaxation is a thermal process in which heat is evolved. However, it is also possible that the molecule may emit a photon in order to dissipate the excess energy. This process is called **fluorescence**. The following figure illustrates the principle of fluorescence as the sole relaxation process.



In this scenario, the molecule is excited into the one of the vibrational levels of the first excited electronic state of the molecule. Next, the molecule vibrationally relaxes to the lowest energy vibrational state of the excited electronic state. This process is known as vibrational relaxation. It is very fast, and the molecule's excess vibrational energy is converted to heat. Following vibrational relaxation, the molecule emits a photon whose energy matches that of the energy gap in one of the downward transitions. It should first be noticed that the energies of the fluorescence photons are actually lower than the absorption photon, making the wavelength of the fluorescence photons higher (more red-shifted) than the excitation photons. If the vibrational level structure in the first excited state is similar to that of the ground state, the absorption spectrum and the emission spectrum will look like mirror images of each other. The frequency between the longest wavelength (lowest energy) excitation line and the shortest wavelength (highest energy) emission line is known as the Stokes Shift (historically, the term Stokes Shift is synonymous with a shift to lower energy, and Anti-Stokes Shift is synonymous with a shift to

higher energy). The excitation wavelength is nearly always shorter than that of the shortest wavelength emission peak, however the separation between the peaks may be very small.

There is a very distinct advantage of fluorescence spectroscopy over direct absorption spectroscopy (UV/Vis). Fluorescence is not a ratiometric technique, and the detected signal is directly and linearly proportional to the concentration of emitting species in the sample, whereas in absorption spectroscopy, the logarithm of the ratio of the transmitted intensity to the incident intensity is proportional to the concentration. Fluorescence is known as a zero-background technique because the signal will be zero when the analyte concentration is zero. As a result, the detection limits of fluorescence are much higher than those for absorption because small concentrations only produce a slight change in the transmitted intensity versus the incident intensity in absorption (which may be ambiguous depending on the stability of the light source, i.e. noise), but produce a nonzero signal in fluorescence (as compared to the background), which is completely unambiguous.

## B. Instrumental Background

Fluorescence spectroscopy uses a continuum excitation source, like that in absorbance spectroscopy, but instead of illuminating the sample with all wavelengths, an excitation monochromator selects out a specific excitation wavelength. This allows only a single excitation transition to occur, and eliminates interferences from the scattering of other wavelengths when detecting emitted intensity from the sample. Therefore, wavelength selection happens twice in a fluorescence instrument, with a monochromator directly after the excitation source, and a monochromator that selects the emitted wavelengths directly after the sample. An excitation wavelength is selected, and is directed onto the sample, and then the emission monochromator scans through the emission wavelengths (usually starting 10 – 20 nm higher than the excitation wavelength), and the intensity at each wavelength is recorded on a stripchart recorder. The resulting spectrum is called an **emission spectrum**. The converse of this process can also be performed, and this spectrum is called the **excitation spectrum**. In this process, the emission monochromator is set to a specific wavelength, and the intensity of emission at the wavelength is monitored as the excitation wavelength is varied. This spectrum should be nearly identical to the absorption spectrum.

### 1: WHY?

#### Cell Care

Spectrophotometric cells must be handled properly if reliable results are to be obtained. A few helpful rules are listed:

1. Inspect for scratches on the windows. Never touch the optical surfaces of cells with your fingers. Finger prints are often fluorescent when excited with UV light.
2. Either use a single cell for an entire series of measurements, or assure that all cells used are transmittance matched for path length.
4. When filling cells, rinse them several times with the solution to be measured, then fill, assuring that no air bubbles adhere to the windows. Wipe the outside of the cell with lens tissue or a Kimwipe, *never* with a paper towel.

5. Place cells into the instrumental carefully, assuring that they are properly seated in the holder. Use the same cell orientation each time.
6. If a volatile solvent is being used, place a cover on the cell to reduce evaporation.
7. Never store solutions in cells. Rinse thoroughly when finished and allow to dry in a dust-free environment. Basic solutions are especially likely to etch cells if they are not properly cleaned.

**NOTE: XENON LAMP IS FIRST ON AND LAST OFF!**

See diagram of instrument at the end of the lab.

### **I. Start-Up**

Set the following settings.

1. SAMPLE SENSITIVITY knob (#23): ZERO
2. AMPLIFIER POWER switch (#27): ON
3. RECORDER POWER switch (#20): AMP
4. FILTER knob (#6) : "s"
5. EXCITATION SHUTTER (#12): CLOSE
6. XENON LAMP POWER SUPPLY (#18): ON  
wait 15 sec  
press start (#17)  
allow to warm for 20 minutes

### **II. Wavelength Calibration**

A fluorescence spectrometer has two monochromators, a light source, and a detector. The light source is directed through one of the monochromators (the excitation monochromator) in order to select a specific band of light to excite the sample. The fluorescence is collected through the second monochromator (the emission monochromator) in order to measure the spectrum of the emitted light. It is good practice to calibrate the monochromators before using the instrument since the wavelength you dial up is seldom exactly what is passed to the sample or to the detector. Normally, some source having a dominant line of known wavelength is used. In our case the xenon lamp, although a continuous source, has numerous strong atomic emission lines, one of which is at 467.1 nm (this is a Hg line; see Figure 3-2 on page 3-5 in the Perkin Elmer manual). This line will be used to calibrate both excitation and emission monochromators according to the following procedure.

- A. Close all shutters (#12, #15), and place the triangular mirror in the optical path such that the excitation beam is reflected to the detector. The mirror should be located in one of the wooden accessory boxes.
- B. Make the following instrument adjustments:  
EMISSION and EXCITATION SLITS (#8,#14): 1.5 nm  
Amplifier REFERENCE SENSITIVITY knob (#24): DIRECT

SAMPLE SENSITIVITY knob (#23): ZERO  
Recorder POWER switch (#20): SERVO  
Scan SPEED: 2 (This is 10 nm/div. Don't change this over the course of the experiment.)  
Amplifier POWER switch (#27): ON

- C. Leaving all the shutters closed, adjust the SAMPLE 0% ADJUST knob (#18) for a zero signal using the meter on the upper left hand corner of the instrument.
- D. Change the SAMPLE SENSITIVITY knob (#23) to 100 (most sensitive setting), and note any changes in the zero position of either the meter or the pen of the chart recorder. If a shift is observed, use the DARK CURRENT knob (#26) and re-zero the instrument. It is preferable to monitor the position of the pen on the chart recorder for this adjustment, as it is far more sensitive than the meter.
- E. Set the SAMPLE SENSITIVITY knob (#23) to 0.1 and the FILTER SELECTOR (#6) to “.” position. Rotate the EXCITATION WAVELENGTH dial (#13) past the lowest wavelength reading (220 nm). A red index mark should be visible on the wavelength dial. Gently line up the red index mark with the cross hair on the window. In this setting, all wavelengths of incoming light are impinged upon the sample.
- F. Adjust the EMISSION WAVELENGTH dial (#9) to 467 nm. Turn the FILTER selector switch to “.” and open the EXCITATION SHUTTER on the front panel. SLOWLY push in the external shutter that lies between the xenon lamp and the spectrometer so that a 50-70% full scale signal is obtained. Opening the external shutter too swiftly will flood the PMT and possibly cause damage. USE CAUTION.
- G. Rotate the EMISSION WAVELENGTH dial (#9) to 400 nm (remember to turn the dial below the desired point and then back up to remove any slack in the mechanism) and scan mechanically to 500 nm at a rate of 10 nm/div (#11). The spectrum obtained should resemble Figure 3-2 on page 3-5 in the PERKIN ELMER manual.
- H. Determine the position of the 467.1 nm line (most intense emission line) according to the wavelength calibration of the instrument. *If a deviation greater than 1 nm exists, use a correction factor to determine wavelength throughout the rest of the experiment.*
- I. The excitation grating can be calibrated in a similar fashion. Turn FILTER to “S” and Emission to 450 nm. Then open the detector (FILTER to “.”) and Manually scan the excitation from 450 to 470 nm. Adjust SENSITIVITY (#23) and EMISSION SHUTTER (#15) as needed to keep the strip chart recorder on scale. Scan the excitation from 450 to 470 nm and determine the position of the 467.1 nm line. *Again, include a correction factor in the rest of your work if the deviation is greater than 1 nm.*

### III. Comparison of Fluorescence and Absorption Spectra

- A. Using the absorption spectrum of perylene in hexane and ethanol which were taken in the UV-Vis Absorption Experiment, determine the best excitation wavelength to excite perylene

fluorescence. Set the excitation monochromator to this wavelength, and collect an emission spectrum of the sample.

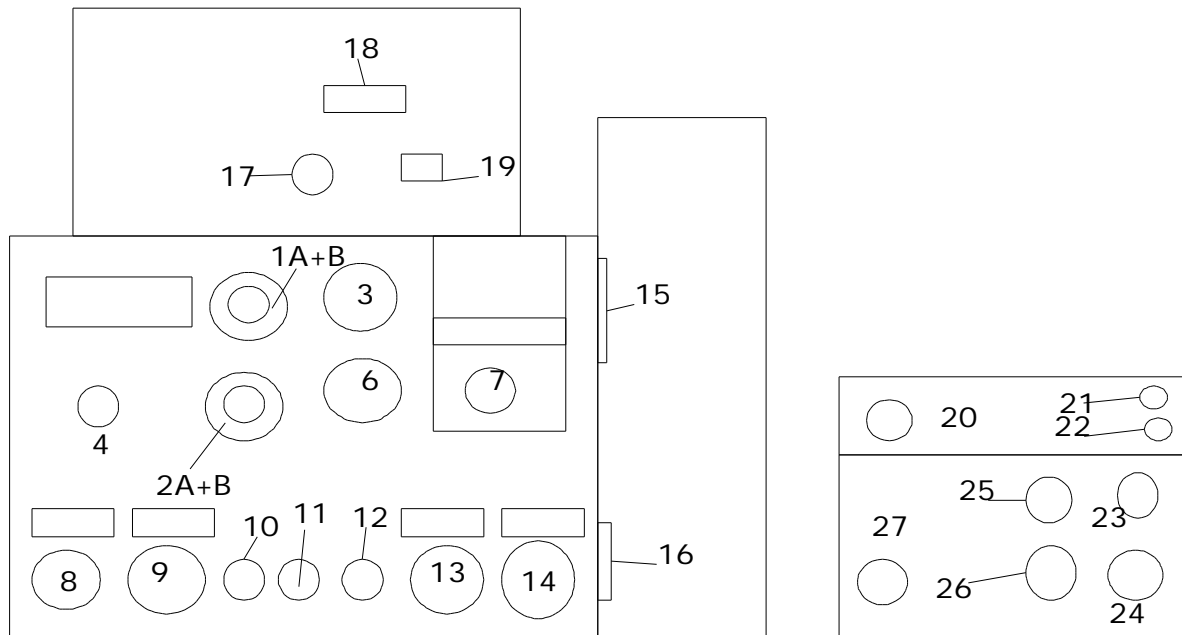
- B. Now set the emission monochromator to the wavelength of the fluorescence maximum, and collect an excitation spectrum.

**2: Compare and comment on the shapes of the excitation, absorption and emission spectra.**

**3: Using corrected wavelengths for both the absorption and emission spectra, measure the stokes shift of perylene in hexane and ethanol.**

- C. Collect emission spectra of the DMABN in hexane and DMABN in ethanol samples for which you measured absorption spectra in the UV-Vis lab. Use excitation wavelengths determined from the absorption spectra. Notice a strong dependence of the emission spectrum wavelengths on the solvent that were not evident in the perylene samples.

**4: Comment on the strong dependence of the fluorescence spectral profile on the solvent.**



- |                               |                               |
|-------------------------------|-------------------------------|
| 1A Sample 100% Adjust Knob    | 13 Excitation Wavelength Dial |
| 1B Sample 0% Adjust Knob      | 14 Excitation Slit Dial       |
| 2A Reference 100% Adjust Knob | 15 External Shutter           |
| 2B Reference 0% Adjust Knob   | 16 Scan Mode Knob             |
| 3 Meter Circuit Selector      | 17 Lamp Start                 |
| 4 Meter Shunt Switch          | 18 Xenon Power Supply On      |
| 5 Meter                       | 19 Power Pressure             |
| 6 Filter Selector             | 20 Recorder Power             |
| 7 Cell Positioning Knob       | 21 Pen Up                     |
| 8 Emission Slit Dial          | 22 Chart Speed                |
| 9 Emission Wavelength Dial    | 23 Sample Sensitivity         |
| 10 Wavelength Drive Knob      | 24 Reference Sensitivity      |
| 11 Scan Speed Knob            | 25 Zero Suppression           |
| 12 Excitation Shutter         | 26 Dark Current               |
|                               | 27 Power                      |

## ***Flame Atomic Absorption and Emission Spectroscopy***

### **I. Background**

Atomic spectroscopy is widely used as an analytical technique for determining the identity of and the amount of metals (generally) in a sample. There are two main types of atomic spectroscopy: atomic absorption and atomic emission. Most atoms have very distinct absorption characteristics, and therefore are easy to identify by the wavelength of light that they absorb.

Flame atomic absorption spectroscopy involves the absorption of light by a vaporized, neutral atomic sample. Flame atomic absorption instruments take an aqueous sample of the analyte and turn it into an aerosol using a nebulizer. The aerosol particles contain metal ions solvated by water. The aerosol is delivered to a flame that desolvates and neutralizes the ions, converting the sample into an atomic gas. A light source that is resonant with the absorption line of the analyte (usually with a very narrow frequency width, or bandwidth) is directed through the flame, and the transmitted intensity of the light is measured. The *ratio* of the transmitted intensity (i.e., light NOT absorbed by the sample) to the incident intensity gives an absorption. Atomic absorption can be a highly sensitive technique, with lower limits of detection reaching the part-per-billion (ppb) range for many analytes.

The excitation source for atomic absorption is the Hollow Cathode (HC) lamp. An HC lamp consists of a glass tube containing a tungsten anode and a metallic cathode usually under an inert gas at a very low pressure (a few torr). The cathodic material is the metal that you wish to analyze. A high potential is applied across the electrodes, which causes some ionization of the inert gases (usually neon or argon). The electric field between the cathode and anode causes the ions to move towards the cathode. If the potential is great enough, some of the gaseous cations may have enough kinetic energy to eject some of the metal atoms on the cathode surface, which causes an atomic gas to form in a process known as **sputtering**. A certain amount of these sputtered atoms are in the excited state when they are ejected, and when they relax, they emit light, which has the same photon energy that is required to excite the metal atoms. Each HC lamp is specially tailored for one and possibly two analytes, and contains a very narrow linewidth excitation source that is exactly the correct wavelength for excitation of the sample. In this lab, we will be using a Calcium/Magnesium HC lamp.

Atomic emission spectroscopy involves the emission of light by atoms in the vaporized atomic sample that are excited due to the thermal energy of the flame and in the absence of HCL excitation source. The amount of excitation that can be attributed to the thermal energy of the flame alone is given in the following expression:

$$\frac{N_j}{N_i} = \frac{G_j}{G_i} e^{\frac{-E_j}{kT}}$$

$N_j$  and  $N_i$  are the number of atoms in the excited state and the ground state, respectively,  $k$  is Boltzmann's constant,  $T$  is the temperature of the flame, and  $E_j$  is the energy gap between the ground and the excited state. The  $G$  terms are known as degeneracies, and they are statistical factors that account of the possibility of more than one state having the same energy. If the excited state formed in the flame has three states (i.e. excitation to a p-orbital), then the  $G_j$  term would be 3, because the  $j^{\text{th}}$  state has a degeneracy of 3. As an example of the use of this calculation, consider the excitation of sodium atoms ( $\lambda = 589.0$  nm) by a 2500 K flame, assuming the ground and excited states have degeneracies of one ( $G_j = G_i = 1$ ). Converting 589.0 nm to energy in Joules ( $E = h\nu = hc/\lambda$ ,  $3.75 \times 10^{-19}$  J), we have  $E_j$ . According to the above expression the ratio of the number of atoms in the excited state to the number of atoms in the ground state is approximately  $5.7 \times 10^{-5}$ , which means that for every 1 atom in the excited state, there are nearly 18,000 atoms in the ground state at this flame temperature.

In practice, atomic emission simply examines the intensity of emitted light at a particular wavelength (selected by an emission monochromator) with respect to background radiation. This technique is inherently more sensitive than absorption, and limits of detection can reach an order of magnitude lower than absorption in most situations.

## II. Instrumentation and Setup

### ALIGNMENT OF THE BURNER HEAD AND HC LAMP

The hollow cathode lamp output must pass over the longitudinal axis of the burner head, and approximately 4 mm above the surface of the burner. This is usually adjusted by adjusting the position of the burner. To align the lamp output properly into the monochromator, the following steps must be taken.

1. First choose the correct analysis by pressing the **LIB** button on the front panel. This will display a screen and at the top will be a library name. For our atomic absorption demonstration we will be examining Mg absorption, and therefore, you must press **SEL** until the proper library is loaded (Mg-D2-285.2-lib3).
2. Press **ESC**. This screen now displays some operating parameters. We must first ensure that the proper lamp is being utilized. The Ca/Mg HC lamp is located in position 1 on the lamp turret. Open the panel on the top left of the instrument, and notice that there are three possible lamp positions. Make sure that the position 1 is on top. Next, notice that the wavelength of detection is 285.2 nm. Turn the wavelength selector on the right side of the instrument to approximately 285 nm. Then notice that the **slit** parameter is 0.7 nm. On the right side of the instrument is a slit width selector. Turn it to the **7 Angstrom** slit width.
3. Now press **ALIGN**. You should see a bar graph appear on the screen, which reflects the amount of energy the PMT is seeing at the wavelength that is selected on the wavelength selector. Adjust the wavelength selector carefully to maximize the power shown on the bar graph. If the bar graph goes off scale in the negative direction, turn the selector back the other way. If the bar graph goes off scale in the positive direction, press the **ALIGN**

button again, and the bar graph will renormalize back on scale. Once you have found the maximum, renormalize once more with the **ALIGN** button.

4. Now you must ensure that the lamp is aligned properly. With the lamp turret panel open, turn the **VERTICAL** and **HORIZONTAL** alignment dials slightly and watch the bar graph on the screen. Maximize the energy with these two adjustments. When the energy is maximized, press **ALIGN** once more to normalize the energy. Now press the **A/Z** (autozero) key.
5. A few seconds after the autozero key is pressed, the active analysis display screen appears, and it displays the absorbance reading. This should remain close to 0.0000, with a fluctuation of  $\pm 0.0020$  being acceptable. If it is more than this, see your TA.
6. Make sure that the aspirator tube (small flexible tube coming off of the burner head) is placed in the ultrapure water container.
7. Go over to the yellow gas tank marked "AIR". Make sure that it is open and that the regulator reads 40 psi. Make proper adjustments to ensure that the pressure stays at 40 psi.
8. Turn oxidant selector on the left side of the instrument from **OFF** to **AIR**. This allows the ultrapure water to be aspirated up through the burner head through the nebulizer. A positive absorbance reading should now be displayed on the screen. Adjust the **HORIZONTAL** knob on the front of the burner head assembly to maximize the absorbance reading.

### **ADJUSTING THE NEBULIZER**

1. With ultrapure water being aspirated, turn the nebulizer adjustment knob, located on the front of the burner head assembly where the aspirator tube meets the main assembly, counterclockwise until the aspirator begins to blow air INTO the ultrapure water container.
2. Now, slowly turn the nebulizer adjustment until it begins to aspirate again. Watch the absorbance readings, which should increase. Find a good balance between high absorbance readings and stability of the absorbance readings.
3. Turn the oxidant selector to the **OFF** position.

### **GASES**

1. Make sure that the **ACETYLENE** regulator reads 10 psi, and the **AIR** regulator reads 40 psi.
2. Flip the **FUEL ON/OFF** switch to the up position, and check the fuel flow meter on the front of the panel. Make sure it reads at least **7**.

3. Flip the **FUEL ON/OFF** switch to the down (off) position.

### IGNITING THE BURNER

1. Turn the oxidant selector to the **AIR** position.
2. Flip the **FUEL ON/OFF** switch to the **UP (ON)** position.
3. Light the lighter just slightly above the slit on the burner head assembly.
4. You should now have a very yellow flame. This signifies that it is **FUEL RICH**. Use the lower **FUEL** knob to decrease the fuel flow, until the yellowish-white part of the flame is just barely visible above the blue portion of the flame near the burner head. This should be at a point where the fuel flow meter reads between **6 and 7**.
5. You will notice that the absorbance reading is probably not 0.0000. Press the **A/Z** key to rezero the instrument.

### TAKING MEASUREMENTS

1. The first measurement to be made is the Magnesium absorption measurements.
  - a. Press the **SMPLS** key.
  - b. Enter the name "Mg Standards" using the **Lower Case and Upper Case** buttons in conjunction with the keypads. Press **ENTER** when the name is entered.
  - c. Press the **DOWN ARROW** button to highlight **Edit Sample Identifiers**, and press **ENTER**.
  - d. You are going to be doing 5 standards of Magnesium, at the following concentrations: 61, 101, 505, 1111, and 2020 parts-per-billion (ppb). On line 1, enter the identifier as **0000**. On lines 2 through 6, enter the following identifiers in this order: "61", "101", "505", "1111", and "2020". Press **ENTER** after each one of these is entered. When you are finished, press the **ESC** key.
  - e. Press the **ESC** key again to get back to the active display screen.
  - f. Now press the **CAL** button on the front panel. Highlight the Display Mode field, which will most likely read **ABSORB/EMISS**. Press the **LEFT ARROW** key so that the word **CONCENTRATION** appears. Press **ENTER**.
  - g. A new set of parameters should appear on the screen. Scroll down with the **DOWN** arrow key and make sure that the **Calibration printing** option is turned **Off**.
  - h. Scroll down to **Enter Calibration Standards** and press **ENTER**.

- i. You should now be at a screen with a sample list at the top, and some parameters on the bottom half of the screen. Highlight the **Units** field and use the left arrow to select **ppb** and press **ENTER**. Then scroll to the **Standard Reps** field and type **3** and press **ENTER**.
- j. Scroll to the **INTGR TIME** field and type in **5.0** in the time field. Press **ENTER**.
- k. Now scroll to the **Edit or Read Data** selection and press **ENTER**.
- l. You should be highlighting selections under the **CUP** column. In the first row of the **CUP** column, type **1** and press **ENTER**. To the right of what you just entered, the numbers "**0000**" should appear. This is the identifier you typed earlier in the **SMPLS** menu.
- m. Scroll down to the next row, and type **1** and press **ENTER**. The number **61** should appear to the right. Continue doing this for cups **2** through **6**.
- n. Now press the **SEL** key to move to the **Concentration** column.
- o. For each **CUP**, type in the concentration of the standard. For **CUP 0**, leave the concentration at 0.0000. For **CUP 1**, type "00061.0000", and hit enter. Continue this for all cups, using the concentrations stated above in ppb. Once all concentrations are entered, press the **ESC** button.
- p. Scroll down and highlight the **AUTO-READ** selection, and press **ENTER**, and then press **ENTER** again (to accept the fact that the autosampler is not set up on this machine).
- q. Now, the cell in row 1 and under the column "Absorbance" should be highlighted. Make sure you are aspirating ultra pure water and press **ENTER**.
- r. Take the 61 ppb standard, and open it. Wipe off the aspirator tube thoroughly with a KimWipe. Place the aspirator tube into the 61 ppb standard, and press **ENTER**. It will now read the absorbance of the 61 ppb standard. At this point, do not change the solution yet, as we are taking three repetitions of each standard, and at the end of the three repetitions, the absorbances are averaged. Press **ENTER** to take the second measurement, and when that is finished, press **ENTER** once more to take the third and final measurement for 61 ppb standard.
- s. Repeat the steps for the other standards, remembering to wipe off the aspirator tube each time you remove it from a sample container.
- t. **RECORD ALL CONCENTRATIONS AND ABSORBANCES IN YOUR NOTEBOOK FOR LATER ANALYSIS.**

- u. Once all measurements are finished, you are automatically returned to the Calibration parameter screen. Scroll down to **Calculate Curve** and press **ENTER**.
- v. Press **YES** to calculate the calibration curve. The coefficient for the linear regression will appear (the slope). **Record this number in your notebook**. Then press **ENTER** to plot the calibration curve. Inspect the graph to ensure linearity.
- w. Press the **ESC** button **three times** to return to the active analysis page. All of the calibration data is kept in the memory, and instead of absorbance units, the active analysis display should display units of **ppb**.
- x. Take a sample of Chemistry Department DI water (from the DI tap) and aspirate it. Press the **READ** button for an analysis of the concentration of Magnesium. **RECORD THIS VALUE AS WELL AS THE ABSORBANCE VALUE (displayed in smaller type on the screen above the concentration value)**.
- y. **REPEAT FOR TAP WATER AND THE UNKNOWN WHICH IS PROVIDED FOR YOU.**
- z. After the lab, plot the results of the calibration standards on a spreadsheet and perform a linear regression analysis on it. **REPORT THE EQUATION OF THE LINE AND THE R<sup>2</sup> VALUE. USE THE EQUATION OF THE LINE TO DETERMINE THE CONCENTRATIONS OF THE WATER SAMPLES AND THE UNKNOWN.**

**DO THE CALCULATIONS MATCH UP WITH THE CONCENTRATION READINGS ON THE INSTRUMENT?**

- 2. The second measurement will involve the atomic emission of Sodium.
  - a. Select the proper library for Sodium Emission, which is titled "Na-589.0-Emis-lib3" from the **LIB** menu.
  - b. Turn the lamp selector turret to position 3 (empty).
  - c. Set the monochromator wavelength selector to the proper wavelength and ensure the slit setting is correct (wavelength approximately 589 nm).
  - d. Aspirate the highest concentration standard of Na (19 ppm). **NOTICE THE HIGH INTENSITY OF ORANGE LIGHT PRESENT IN THE FLAME. THIS IS THE SODIUM EMISSION AT 589 NM.**
  - e. Press the **ALIGN** button to display the bar graph and adjust the wavelength selector to maximize the signal. Once the signal is maximized, press the **ALIGN** button to renormalize the energy, and then press the **ESC** button to return to the active display screen.

- f. While still aspirating the 19 ppm sample, press the **RSLP** key (the reslope key). This resets the PMT voltage so that the highest concentration sample will read 100.000 on the emission scale.
- g. Aspirate each of the three samples of Na, and record the concentrations and emission values. The instrument does not perform calibrations for the atomic emission analysis. Therefore, you will need to form a calibration plot in your spreadsheet of choice, and **REPORT THE LINEAR EQUATION AND THE  $R^2$  VALUE**. It may not be a great calibration plot, since only three points are being used.
- h. Aspirate DI water and tap water, and using your calibration plot, determine the concentration of sodium in the water. The tap water may need to be diluted to put it in the range from 1 – 100 emission units. **REPORT THESE VALUES IN YOUR LAB REPORT.**

### **SHUTDOWN PROCEDURE**

1. Make sure that you are aspirating ultrapure water.
2. Flip the **FUEL ON/OFF** switch to the off position. The flame will extinguish.
3. Wait about 10 seconds, and then turn the oxidant selector to the **OFF** position.
4. Turn the instrument display and lamps off with the power switch, located on the right side of the back panel.
5. Shut off all gas tanks.