EXPERIMENT 18
SPECTROPHOTOMETRIC DETERMINATION OF AN EQUILIBRIUM CONSTANT

INTRODUCTION

Some of the most important and interesting chemical systems, including solutions of slightly soluble salts or of transition metal complexes, are examples of chemical equilibria. Perhaps the most extensively studied of all equilibrium systems are the reactions of weak acids and bases. For example, a weak acid HA reacts with water to establish the following equilibrium:

\[
HA + H_2O \rightleftharpoons A^- + H_3O^+ \quad (18-1)
\]

The equilibrium constant for this acid dissociation reaction, \(K_a\), is expressed as

\[
K_a = \frac{[A^-][H_3O^+]}{[HA]} \quad (18-2)
\]

An indicator is a weak acid or base that can be used to locate the endpoint of a reaction between acidic and basic solutions. Indicators are themselves weak acids or bases that exhibit one color in their acid form and another in their conjugate base form. For instance, the indicator bromothymol blue is yellow in its acid form (abbreviated HIn) and blue in its conjugate base form (abbreviated In\(^-\)). The equilibrium between these species is

\[
HIn + H_2O \rightleftharpoons In^- + H_3O^+ \quad (18-3)
\]

yellow \hspace{1cm} blue

\[
K_{In} = \frac{[In^-][H_3O^+]}{[HIn]} \quad (18-4)
\]

Indicator concentrations are kept very low compared to the concentrations of other acids or bases present, so the equilibrium in EQUATION 18-3 has almost no effect on the total \(H_3O^+\) concentration of the solution. This is determined instead by the other acids or bases present in much higher concentration, through equilibria such as in EQUATION 18-1. EQUATIONS 18-3 and 18-4 still apply, however, and the result is that the value of the \(H_3O^+\) concentration, together with \(K_{In}\), determines [HIn] and [In\(^-\)]. This fact makes indicators useful for revealing pH changes.

Rearranging EQUATION 18-4 gives

\[
\frac{[In^-]}{[HIn]} = \frac{K_{In}}{[H_3O^+]} \quad (18-5)
\]

which shows how the ratio of the concentrations of the two forms of the indicator depends on both \([H_3O^+]\) and \(K_{In}\). The logarithmic form of this equation, after some rearrangement, and using the definitions of pH and pK, is
\[
\log \frac{[\text{HIn}]}{[\text{In}^-]} = -\ p\text{H} + pK_{\text{In}} \tag{18-6}
\]

In this experiment, measurements on a series of solutions of an indicator with different pH values will enable you to determine \(pK_{\text{In}}\) (and, therefore, \(K_{\text{In}}\)) for the indicator.

**TECHNIQUE AND ANALYSIS**

If the concentrations of all the products and reactants in the equilibrium system could be measured, it would be a simple matter to insert these values in **EQUATION 18-4** to obtain \(K_{\text{In}}\). Unfortunately, it is rarely possible to experimentally determine all of the concentrations for an equilibrium system. In this experiment, you will use an indirect method that makes it unnecessary to measure the actual concentration of each component. (This situation provides yet another illustration of a common occurrence in an experimental science. When we cannot directly measure what we wish to know, appropriate mathematical manipulations, admittedly rather messy and involved in some cases, allow us to establish a convenient connection between what we can measure and what we wish to determine. The result in this case is a relatively simple expression, **EQUATION 18-18**, which we use to extract the desired quantity. It will greatly increase your understanding of this experiment if you follow through the derivation below with pencil and paper, verifying each step in the process.)

Since we are dealing with colored materials, we can take advantage of the MeasureNet’s spectrometer to measure visible absorption spectra to make our equilibrium constant determination. You will record the entire visible spectrum for each of a series of samples and then find the absorbance values for a chosen wavelength.

Over a pH range that includes its \(pK_{\text{In}}\) value, an indicator can exist solely as HIn (at any pH well below \(pK_{\text{In}}\)), solely as In\(^-\) (at any pH well above \(pK_{\text{In}}\)), or as some mixture of HIn and In\(^-\) at intermediate pH values. To simplify our analysis, we will keep the total concentration of indicator, \(T_{\text{In}}\), the same in all solutions:

\[
T_{\text{In}} = [\text{HIn}] + [\text{In}^-] \tag{18-7}
\]

The absorbance, \(A_{\lambda}\), of a sample at some wavelength, \(\lambda\), can be expressed according to Beer’s Law:

\[
A_{\lambda} = \varepsilon_{\lambda} \cdot b \cdot C \tag{18-8}
\]

where \(\varepsilon_{\lambda}\) is the molar absorptivity at the chosen wavelength, \(b\) is the length of the light path through the sample, and \(C\) is the molar concentration of the absorbing species. For a solution containing a mixture of HIn and In\(^-\), the total absorbance is the sum of the contributions from the two species:

\[
A_{\lambda} = \varepsilon_{\text{HIn}} \cdot b \cdot ([\text{HIn}]) + \varepsilon_{\text{In}^-} \cdot b \cdot ([\text{In}^-]) \tag{18-9}
\]

For convenience, we define the absorbance of this mixture alternatively in terms of an apparent molar absorptivity (\(\varepsilon_{T_{\text{In}}}\)) of the total concentration of indicator:

\[
A_{\lambda} = \varepsilon_{T_{\text{In}}} \cdot b \cdot T_{\text{In}} \tag{18-10}
\]

Combining **EQUATIONS 18-9** and **18-10** and eliminating \(b\), we obtain

\[
\varepsilon_{T_{\text{In}}} \cdot T_{\text{In}} = E_{\text{HIn}} \cdot [\text{HIn}] + \varepsilon_{\text{In}^-} \cdot [\text{In}^-] \tag{18-11}
\]
Note that, even though $\varepsilon_{HIn}$ and $\varepsilon_{In^-}$ are constants for the chosen wavelength, $\varepsilon_{In}$ is not a constant. It varies from one solution to the next as $[HIn]$ and $[In^-]$ change. If Equation 18-7 is rearranged to give $[In^-] = T_{In} - [HIn]$ and this is inserted into Equation 18-11, the result can be written

$$[HIn] = T_{In} \cdot \frac{\varepsilon_{T_{In}} - \varepsilon_{In^-}}{\varepsilon_{HIn} - \varepsilon_{In^-}}$$  \hspace{1cm} (18-12)$$

Similarly, if Equation 18-7 is rearranged to give $[HIn] = T_{In} - [In^-]$, substitution into Equation 18-11 yields

$$[In^-] = T_{In} \cdot \frac{\varepsilon_{HIn} - \varepsilon_{T_{In}}}{\varepsilon_{HIn} - \varepsilon_{In^-}}$$  \hspace{1cm} (18-13)$$

Dividing Equation 18-12 by Equation 18-13 gives us

$$\frac{[HIn]}{[In^-]} = \frac{\varepsilon_{T_{In}} - \varepsilon_{In^-}}{\varepsilon_{HIn} - \varepsilon_{T_{In}}}$$ \hspace{1cm} (18-14)$$

This can be inserted into Equation 18-6 to give

$$\log_{10} \frac{\varepsilon_{T_{In}} - \varepsilon_{In^-}}{\varepsilon_{HIn} - \varepsilon_{T_{In}}} = -pH + pK_{In}$$ \hspace{1cm} (18-15)$$

In this experiment, you will generate absorption spectra for an indicator at various pH values. At low pH (high $[H_3O^+]$), the indicator exists only as HIn, since the equilibrium in Equation 18-3 is shifted far to the left (i.e., $[HIn] = T_{In}$ at low pH). Therefore, the absorbance at low pH, $A_L$, is

$$A_L = \varepsilon_{HIn} \cdot b \cdot T_{In}$$ \hspace{1cm} (18-16)$$

Similarly, at high pH, the indicator exists only as In$^-$ ($[In^-] = T_{In}$ at high pH), and the absorbance at high pH, $A_H$, is

$$A_H = \varepsilon_{In^-} \cdot b \cdot T_{In}$$ \hspace{1cm} (18-17)$$

At intermediate pH values we have Equation 18-10. Using Equations 18-10, 18-16 and 18-17 to substitute for $\varepsilon_{HIn}$, $\varepsilon_{HIn}$, and $\varepsilon_{In^-}$ in Equation 18-15, followed by elimination of $b \cdot T_{In}$, yields our final equation:

$$\log_{10} \frac{A - A_H}{A_L - A} = -pH + pK_{In}$$ \hspace{1cm} (18-18)$$

Note that we have indeed, through this rather long process, succeeded in deriving an expression containing only quantities we can measure and the quantity we are trying to determine. To make use of this expression, we take the quantity on the left-hand side of the equation as $y$ and the pH as $x$, obtaining the equation for a straight line: $y = mx + b$. You will choose an appropriate wavelength for the indicator, determine the absorbance, $A$, at this wavelength for each of a series of pH values, and produce the plot just described. The values of $A_H$ and $A_L$ will come from the extremes in this series (highest and lowest pH values). There will be one point in the plot for each intermediate pH value. Examination of Equation 18-18 shows that this straight-line plot should have a slope of $-1$ and a y-intercept of $pK_{In}$. Further examination shows that the intercept on the pH axis is also equal to $pK_{In}$. (i.e., when the left-hand
side (y) of the equation = 0, pH = \( pK_{In} \). It will be preferable, and easier, to determine \( pK_{In} \) from the intercept on the pH axis, since this intercept will lie somewhere in the middle of the experimental points. To determine the y-intercept requires an extrapolation from the measured values.

**PROCEDURE DIAGRAM**

This experiment involves carrying out a number of steps in sequence, all of which must be completed correctly if good results are to be obtained. You may find it helpful to refer to the following diagram to keep the overall structure of the experiment clearly in mind.

- **Preparation of Buffers**
  - calibrate pH electrode
  - adjust stock buffer to desired pH values

- **Preparation of Indicator Solution**
  - dilute 5.00 mL of indicator stock solution to 25.00 mL with buffer solution

- **Measurement of Spectrum**
  - zero, reference, sample

- **Analysis of Combined Spectra**
  - upload spectra to database
  - access database from external computer and download both your spectra and an Excel workbook to use in the analysis
  - produce and print out an overlay plot of absorption spectra for each solution
  - produce and print out a plot of absorbance

Repeat for each of the buffer solutions.
**EQUIPMENT NEEDED**

- 50-mL beaker
- 150-mL beaker
- 400-mL beaker
- dropping bottles (4)
- magnetic stir plate
- magnetic stir bar
- labels
- MeasureNet
- pH electrode

<table>
<thead>
<tr>
<th>Beaker</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00-mL volumetric pipet</td>
<td>pipet pump</td>
</tr>
<tr>
<td>10.00-mL volumetric flask</td>
<td>disposable cup</td>
</tr>
<tr>
<td>disposable plastic pipets (11)</td>
<td>medium test tubes (7)</td>
</tr>
<tr>
<td>wash bottle</td>
<td>MeasureNet spectrometer</td>
</tr>
<tr>
<td>cuvets (2)</td>
<td></td>
</tr>
</tbody>
</table>

**CHEMICALS NEEDED**

- indicator solutions
- buffer solutions: pH = 4.00, 7.00, 10.00
- 1M HCl solution
- 6M HCl solution
- 1M NaOH solution
- 6M NaOH solution

**PROCEDURE**

**Logging In**

In this experiment, you will save your data as you normally do. However, by logging in at your workstation prior to the collection of any data, not only will the data be stored on the network computer, but it also will be uploaded to Chem21labs.com. You will then be able to download the data from any remote computer with internet access, and analyze the data using Excel.

In order to send and properly store your data at the website, you must first log in at your workstation. Press the MAIN MENU button. Press the function key listed for OTHER, and then the function key for LOG IN. When prompted, enter the experiment number (18) and your 4-digit student ID number that you obtained from the website before you came to your lab room. If you are working with a lab partner, he or she must also log in. This can be accomplished by pressing F1 and following the procedure described above. Once you leave the laboratory, the data will only be accessible to those students who have successfully logged in.

**Calibrating the Electrode**

The pH electrode is essentially an electrochemical cell that generates a small signal (millivolt range) when placed in contact with an aqueous solution containing hydronium ions. The voltage is dependent upon the concentration of H$_3$O$^+$ and therefore upon pH. (You can learn more about electrochemical cells from your textbook.) Before a pH reading is made on a test solution, the electrode must be calibrated with a buffer solution of known pH. A pH electrode is a delicate and fairly expensive piece of equipment. Exercise caution when handling the electrode. The tip of the electrode may be protected by a black plastic electrode shield—if so, do not remove this shield for any reason.

Obtain pH = 4.00 standard buffer solution in a disposable plastic microbeaker. Secure the electrode to a ring stand using a universal clamp and lower the electrode into the microbeaker containing the buffer solution. Press
**MAIN MENU** on the MeasureNet workstation. Since we just wish to use the station as a pH meter for now, select **pH/MV** from the menu, then either one of the two pH options (**pH V TIME OR pH V VOLUME**) that appear next. Select **CALIBRATE**—the workstation will prompt you to enter the temperature (enter ‘22’ for the temperature; it is not important to know the exact temperature for this experiment) and the known pH of the standard solution, and then to monitor the displayed pH of the standard solution. Once the pH reading has become steady, press **ENTER**. Pour the pH = 4.00 buffer into a medium test tube (you’ll need it later on), dry the microbeaker, and refill it with the pH = 10.00 buffer solution. Rinse the pH electrode with distilled water and place it in the pH = 10.00 buffer solution. Follow the prompts on the workstation screen to complete the two-point calibration. After calibration, with the electrode still in the standard solution, press **DISPLAY** and note the pH value indicated on the workstation display. If it is not within 0.02 pH units of the known pH of the standard solution, you should redo the calibration. Save the pH = 10.00 buffer solution in another medium test tube.

**Preparing the Buffer Solutions**

Your instructor will assign you an indicator solution to investigate. Obtain ~20 mL of the indicator solution in a clean, dry 50 mL beaker and ~60 mL of the pH = 7.0 stock buffer solution in a 150 mL beaker. Also obtain the 1M and 6M HCl and NaOH solutions in 4 dropping bottles (you should only need to fill them about ½ full), labeling them so that they don’t get mixed up. Use the table below to determine which indicator to use and the pH values of the buffer solutions you should prepare.

<table>
<thead>
<tr>
<th>Workstation #</th>
<th>Indicator</th>
<th>pH Values of Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 4, 7, 10</td>
<td>Bromocresol purple</td>
<td>5.4, 5.7, 6.0, 6.3, 6.6</td>
</tr>
<tr>
<td>2, 5, 8, 11</td>
<td>Bromothymol blue</td>
<td>6.4, 6.7, 7.0, 7.3, 7.6</td>
</tr>
<tr>
<td>3, 6, 9, 12</td>
<td>Phenol red</td>
<td>7.1, 7.4, 7.7, 8.0, 8.3</td>
</tr>
</tbody>
</table>

Place ~10 mL of the pH = 7.0 stock buffer solution in a 50-mL beaker along with a stir bar. Set the beaker on a stir plate and turn the stir plate dial to the lowest setting. Using a wash bottle, rinse the electrode with some distilled water into a large waste beaker. Lower the electrode into the buffer solution, being careful to avoid letting the spinning stir bar hit the electrode. Monitor the pH on the workstation screen. Adjust the pH of the solution to the lowest target value (±0.05) for your indicator according to the above table by adding the 1M HCl solution dropwise. Record the final pH value, then pour the solution into a clean, dry medium test tube. Label the test tube.

Repeat the procedure to prepare the remaining solutions, using 1M HCl if you wish to lower the pH of the stock buffer solution and 1M NaOH if you wish to raise the pH.

Add two drops of the 6M HCl solution to the test tube containing the pH = 4 buffer. It is not important that we know the exact pH of this solution, only that it is acidic enough so that the indicator is fully protonated when in this solution. Pour the solution into a labeled test tube.

Add two drops of the 6M NaOH solution to the test tube containing the pH = 10 buffer. It is not important that we know the exact pH of this solution, only that it is basic enough so that the indicator is fully deprotonated when in this solution. Pour the solution into a labeled test tube.
Preparing the Indicator Solutions

Rinse the volumetric flask with distilled water. It is not necessary to ensure that it is completely dry; a small amount of water will not affect the pH of a buffer solution. Rinse the pipet with distilled water, then with a small amount of the indicator solution.

Pipet 2.00 mL of the indicator solution into the volumetric flask. **Note:** In order to obtain the same total concentration of indicator in each solution, it is important that great care is taken in delivering a precise volume of solution. Make sure that the bottom of the solution meniscus is just touching the mark on the pipet, and wipe off the outside of the pipet with a paper towel before emptying the solution into the flask. Do not try to force any solution remaining in the tip of the pipet into the flask; the pipet is calibrated to account for this remaining volume.

Fill the volumetric flask to the mark with the buffer solution of lowest pH (pH <4). Use a disposable pipet to deliver the final portion of solution dropwise. Again take care to align the bottom of the meniscus with the mark on the flask. Cap the flask and mix the solution thoroughly by inverting the flask several times. Observe and record the color. If there is any buffer solution remaining in the test tube, discard it.

Setting Up the Workstation

Press the **MAIN MENU** button on the workstation, select **SPECTROSCOPY**, then **ABSORPTION**(1), and press **DISPLAY**.

Press **SETUP** and set the absorbance range at 0–1.5 by entering the value 1.5 at the blinking cursor. Press **ENTER**, then **DISPLAY**. Leave the other graphing limits at their default values.

Rinse and then fill one of the cuvets with distilled water. This will be your reference, or blank cell, to be used in each absorption measurement. Rinse the other cuvet with a small amount of your buffered indicator solution, then fill it to within ~1 cm of the top. Pour the remaining indicator solution into the empty test tube that held the buffer for this sample. (We wish to save this solution in case a spectrum needs to be re-measured later.) Take the cuvets to the spectrometer.

Obtaining Absorption Spectra

At the spectrometer, press **STATION NUMBER**, your station number, then **ENTER**.

Place the reference cuvet (containing distilled water) in the sample holder. Be sure that the side of the cuvet with an arrow at the top is facing toward the light source and that the cuvet is seated snugly in the sample holder. Block the light path by inserting the black shutter, and press **ZERO** on the spectrometer. This zeroes the spectrometer: it measures and records any small signals from the spectrometer in the absence of light so that they can be subtracted from subsequent measurements.

Remove the shutter to allow light to pass through the reference cell into the spectrometer, then press **REFERENCE** on the spectrometer.

Remove the reference cuvet from the sample holder and replace it with your cuvet containing the indicator solution, making sure that the arrow on the cuvet is aligned properly. Press **SAMPLE** on the spectrometer.

Remove your cuvet and return to your workstation.

When the absorption curve appears on the screen, press **FILE OPTIONS** then select **SAVE** to save the data to a file on the computer and to upload it to the web data storage site. Enter **001** for the three-digit file code for the
spectrum and then press **ENTER**. Check with your instructor to ensure that the data has been uploaded properly. **Note:** You do not print any spectra while in the lab room; you will prepare your plots from a remote computer at a later time. Do not clear the workstation screen after each spectrum is plotted—that way you can see all the spectra overlaid on the same display.

Rinse the volumetric flask with a small portion of the buffer solution with the second lowest pH, pipet 2.00 mL of the indicator solution into the flask, fill to the mark with the buffer, and mix. Observe and record the color of the solution. Empty the contents of your sample cuvet in the sink, rinse it with a small portion of the new solution, and refill the cuvet with this solution. Remember to save the leftover buffered indicator solution in a test tube. Return to the spectrometer and obtain a spectrum for this sample. When you return to your workstation, compare the spectrum of this solution with that of the previous solution. Which portions of the spectrum are increasing in absorbance? Which are decreasing? Save this spectrum, using file code **002**.

Repeat this process with the remaining solutions in order of increasing pH. Save each file with consecutively increasing file codes (**003**, **004**, etc.) so that the pH > 10 solution is saved with a file code of **007**. After saving each spectrum, check with your instructor to ensure that the data has been uploaded properly.

You should check the workstation screen after each spectrum is obtained in order to ensure that they have been done properly. You should obtain something similar to **Figure 18-1**, although the details will depend on which indicator you are studying. If all of the manipulations have been carried out properly, all of the spectra should have the same baseline absorbance at 700 nm and higher, and there should be one or more points on the plot where all seven solutions have the same absorbance value. Such a point is known as an isosbestic point, and there is a clear example near 500 nm in **Figure 18-1**. The overlaid plots may also be viewed using the Absorbance Series program on the computer. If one of your absorbance curves does not go through the isosbestic point(s) in your plot, then some error was made, either in preparing the solution or in measuring its spectrum. Identify the solution in question (your instructor can help you do this), and decide whether you need to remeasure its spectrum or prepare a

![Figure 18-1. Absorption Spectra of an Indicator at Various pH Values](image-url)

Figure 18-1. Absorption Spectra of an Indicator at Various pH Values
new solution and generate a new absorption curve. Save any new file with the same file code you used previously (to overwrite and replace the old file). Consult your instructor for help in deciding which curve(s), if any, need to be redone. Once you are confident that your spectra have been obtained and uploaded properly, discard any excess solution down the sink. Rinse all glassware with distilled water before returning

**Obtaining Absorbance Values**

Log on to Che21 and access the exp. 18 report sheet. After you have entered and confirmed the code number for your assigned indicator, click on the Graph Data link to view a plot of all of your absorbance spectra. Select a wavelength at or near the absorbance maximum for the file 007 (pH>10) spectrum, and determine the absorbance for each spectrum at that wavelength. Use the data to prepare the plot below.

**Preparing log f(A) vs. pH plot using Excel**

1. Type the following column headings in cells A1-E1:
   A: file number
   B: pH
   C: Absorbance
   D: f(A) (this represents the function of A: \((A-A_H)/(A_L-A))\) as seen in EQUATION 18-18.
   E: log f(A) (this represents the log of the above function)
2. Change the width of each column to 12.00, and center the data.
3. In column A, enter the file numbers 1 through 7 (they don’t have to be written as 001, 002, etc.).
4. In column B, enter the pH values that correspond to each file number. **Use <4 and >10 for the low and high pH values, respectively.**
5. In column C, enter the absorbance values you obtained from the plot on Chem21 that correspond to each pH (Note: the absorbance values should correlate with the pH values—the higher the pH, the larger the absorbance).
6. Select all of the data, including the column headings. From the Data menu on the Menu bar, select ‘Sort…’. Choose ‘pH’ from the ‘Sort by’ box and hit OK. Your high and low pH data should now be at the bottom of the data table.
7. In cell D2, type \(=(C2-C\$8)/(C\$7-C2)\) (note: don’t forget the parentheses). Drag and copy this equation to the next fours cells (rows 3-6) in the D column.
8. In cell E2, type \(=\text{log}(D2)\). Drag and copy this equation to the next fours cells (rows 3-6) in the E column.
9. Click on cell B1, drag down to B6, then while pressing the Ctrl key, click on E1 and drag down to E6. This will highlight the data you will be plotting.
10. Select Insert from the menu bar, and prepare a scatter plot.
11. From the Chart menu, select Trendline, then Linear Trendline.
12. **From the plot, determine the pK_in value!**
**DATA SUMMARY TABLE**

Indicator Used: ______________

Wavelength Used for Analysis: _________ nm

<table>
<thead>
<tr>
<th>pH</th>
<th>Color of Solution</th>
<th>$A$</th>
<th>$(A-A_H)/(A_LA)$</th>
<th>$\log[(A-A_H)/(A_LA)]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>low (&lt;4)</td>
<td></td>
<td>$A_L$</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>high (&gt;10)</td>
<td></td>
<td>$A_H$</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

$pK_{In} =$ ___________________ $K_{In} =$ ___________________
Notes—Experiment 18
Spectrophotometric Determination of an
Equilibrium Constant

Helpful Hints:

- Before you begin obtaining any absorbance spectra, check with your TA to make sure you have been logged in properly.

- Do not leave the lab room until you and your TA have made sure that your spectra have been obtained properly and that all seven data files have been uploaded to the website.

Calibrating the pH Electrode:

- Use the disposable plastic cups provided.

Preparing the Buffer Solutions:

- It is not crucial that you adjust the pH of the stock buffer solution to exactly the target pH value. Anything ±0.05 of the target value is OK—however, you should record the actual pH and enter this value when you make your plot at the end of the experiment.

Preparing the Buffered Indicator Solutions

- Be very careful when measuring your volumes or you will have to do them again later when you find your spectra are not acceptable.

- Be sure to set up your MeasureNet workstation before you go to obtain your first spectrum.

Obtaining the Spectra

- Important: be sure to enter your station number at the spectrometer each time before you record a spectrum.

- Make sure the cuvet is aligned properly (arrow toward light source) and seated snugly before each measurement.

- Use the correct (001, 002, etc.) 3-digit file code for each spectrum that you save!

- Do not throw out your solutions until you are sure you have obtained satisfactory spectra!