

# Spectrophotometric Analysis of *trans*-Co[(en)<sub>2</sub>Cl<sub>2</sub>]Cl and the Aquation Product, [Co(en)<sub>2</sub>(H<sub>2</sub>O)Cl]Cl<sub>2</sub>

In the spectrophotometric portion of this experiment you will analyze *trans*-[Co(en)<sub>2</sub>Cl<sub>2</sub>]Cl that you previously prepared and its acid catalyzed hydrolysis product, [Co(en)<sub>2</sub>(H<sub>2</sub>O)Cl]Cl<sub>2</sub>.

You will compare the visible absorption spectra of the two cobalt complexes in aqueous solution, examine how their separate absorptions depend on the concentrations of the cobalt complex solutions, and use the combined absorptions to determine the concentrations of the two cobalt complexes in an aqueous mixture.

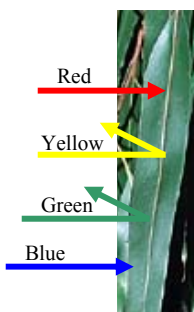
## A Brief Introduction to Spectrophotometry

The use of spectral data to identify and quantify substances is fundamental to chemical analysis. You are already familiar with the absorption spectra that are produced when atoms of an element absorb photons of electromagnetic radiation of certain wavelengths and cause an electronic transition from lower to higher energy states. You learned in Chemistry 2A that each element has a characteristic absorption spectrum.

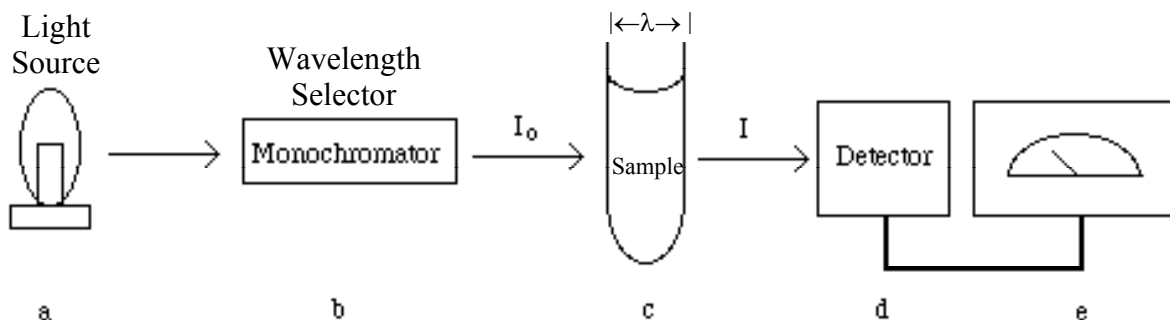
Many substances absorb photons in the visible light region of electromagnetic radiation spectrum while reflecting other photons in the visible light region. As a result of the reflected photons, a particular substance appears a certain color. Visible light, therefore, may be used to study colored substances. This is referred to as spectrophotometry. For example, a blade of grass is green, because its chlorophyll absorbs red and blue wavelengths strongly and green less strongly, so most of the green is reflected, as shown by Figure 1 below.

The height and shape of the curve make up a characteristic absorption spectrum for a substance and can be used for identification purposes. The curve varies in height because chlorophyll absorbs incoming wavelengths to different extents. The absorption spectra of chlorophyll *a* and chlorophyll *b* is shown below. There is high absorption of red and blue frequency photons,  $h\nu$ .

**Figure 1. The absorption spectra of chlorophyll *a* and chlorophyll *b*.**



A spectrophotometer (or spectrometer, for short) is used to measure absorption spectra and is a combination of a light source that emits a continuous band of radiation, a monochromator (usually a reflection grating with associated optics) to select a narrow range of wavelengths, and a photoelectric detector to measure the light intensity, as indicated in Figure 2.



**Figure 2: Schematic Representation of a Spectrophotometer.**

In addition to identifying a substance, an absorption spectrum can be used to determine its concentration because the absorbance, the amount of light of a given wavelength absorbed by a substance, is proportional to the number of molecules present in the sample. This relationship is called the Beer-Lambert Law or more simply Beer's Law. Consider monochromatic light of a given intensity incident on a sample, as shown in Figure 2. If this light can be absorbed by the sample, then the transmitted light will have a lower intensity than the incident light. The transmittance,  $T$ , is defined as the ratio of the transmitted intensity,  $I$ , to the incident intensity,  $I_0$ ,

$$T = \frac{I}{I_0}$$

The percent, %T, is simply  $100 \cdot T$ . The transmittance is decreased if either the concentration,  $C$ , of absorbing substance is increased or the path length,  $\lambda$ , of the sample is increased, since both increase the number of absorbing species in the path of the light.

A related quantity is the absorbance,  $A$  which is given by

$$A = -\log T = -\log_{10} \frac{\%T}{100} = 2 - \log_{10}(\%T)$$

%T is read directly from the spectrometer. However, the absorbance is particularly important since it, and not the transmittance, is directly proportional to the concentration of the absorbing substance and the path length. This proportionality constitutes Beer's Law, and is commonly written as

$$A = \epsilon \lambda C.$$

The concentration,  $C$ , is expressed in moles per liter (M) and the path length,  $\lambda$ , is generally expressed in cm. The quantity  $\epsilon$  is called the molar absorptivity and has units of  $M^{-1} \text{ cm}^{-1}$ . Note that the quantity  $\epsilon\lambda C$  is dimensionless, as are both the absorbance,  $A$ , and the transmittance,  $T$ . The molar absorptivity is characteristic of the substance. It tells us how much light the substance absorbs at a particular wavelength. A graphical plot of either the absorbance at constant path length or the molar absorptivity versus wavelength is called the absorption spectrum of the substance.

Beer's Law forms the basis for the analytical use of spectroscopy to determine concentrations. As indicated by the equation of Beer's Law shown above, a plot of the absorbance at a given wavelength for a particular species versus the concentration of the species yields a straight line with a slope equal to  $\epsilon\lambda$  and an intercept of zero. Since the path length of the cell used for the absorbance measurements is typically known, the molar absorptivity of the species at a chosen wavelength is readily determined from such a Beer's Law plot. The concentration of the species in an unknown sample can then be determined by measuring the absorbance of the sample at the same wavelength in any cell of known path length.

The wavelength for such an analysis should be chosen so that small changes in wavelength do not yield large changes in absorbance. Namely, the chosen wavelength should be in a relatively flat portion of the absorption spectrum. Typically, a wavelength associated with a maximum in the spectrum is chosen, since at a maximum, the slope of the spectrum is zero or horizontal and simultaneously good sensitivity is obtained in the analysis (significant absorbance for a given concentration).

One should always establish, before its analytical use, that Beer's Law is followed by a species over the concentration range of interest, since deviations from Beer's law often occur at high concentrations. Typically, these deviations can be traced to changes in the absorbing species or the bulk solution with concentration. For example, in concentrated solutions the solute molecules are closer together on average and interact with each other, changing their energy levels and spectroscopic properties from a dilute solution. The species of interest also may exist in equilibrium with other species that have different molar absorptivities. In such cases, a graph of absorbance versus concentration will appear to deviate from a straight line at high concentrations.

If two species are present, and neither affects the light absorbing properties of the other, then the observed total absorbance is simply the sum of the absorbances of the individual species. When this is true, the individual concentrations can be determined from spectrophotometric measurements. Because interactions often do arise, sometimes when least expected, the absorption spectra of the species should be investigated when they are separate and when they are simultaneously present to determine whether the absorbances are indeed additive before any analytical spectrophotometric measurements.

## **Operation of GENESYS™ 20 Spectrophotometer**

Spectrophotometers all contain some kind of light source (visible instruments often use just a tungsten bulb); various mechanical and optical elements which are used to create a directed and collimated beam; a wavelength selector of some sort (often either a diffraction grating or a prism); an enclosed cell compartment to hold the absorbing sample; a radiation detector (often a photocell or more commonly now a photodiode); and some kind of readout device (e.g. a needle deflection on a meter).

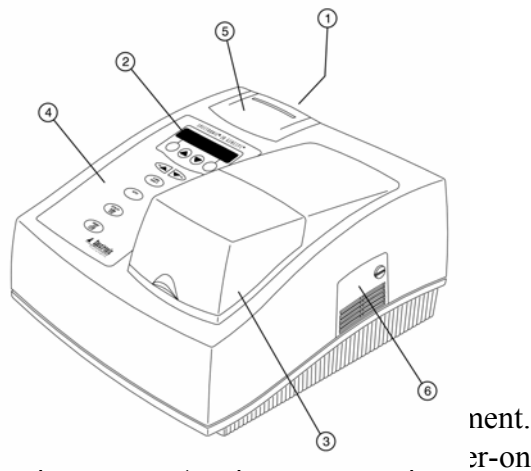
It is important that you understand the operation of the GENESYS™ 20 before you begin this laboratory. The GENESYS™ 20 is an expensive and sensitive instrument and must be operated carefully and intelligently.

The light source in a GENESYS™ 20 is an ordinary tungsten lamp whose radiation extends over the entire visible range. The light from the lamp passes through an entrance slit and is dispersed by a diffraction grating. The grating can be rotated so that a small band of selected wavelengths from the dispersed beam passes through an exit slit, and then through the cell (cuvette) containing the sample. The cuvettes used with a GENESYS™ 20 have a path length (internal diameter) of 1.00 cm. The light transmitted through the sample strikes a solid-state silicon detector that generates an electrical signal proportional to the radiant power (light intensity). The signal from the detector drives a meter that can be calibrated to read transmittance or absorbance.

The *calibration procedure* entails setting 0 Absorbance at a given wavelength with a cuvette containing a reference or blank solution. The blank solution is missing the component of interest, but is otherwise as identical as possible to the solution to be analyzed for the component of interest. Typically, the blank solution is just the solvent. This is required since the output of the lamp and the sensitivity of the detector varies with wavelength. The electronics of the instrument automatically sets 100% absorbance. An identical cuvette containing the solution of interest is then inserted into the spectrometer, and the absorbance is read from a meter on the instrument. Both the calibration and the reading must be done at the same wavelength. The reading for the solution then represents the absorbance at the chosen wavelength due to the component of interest. The calibration has accounted for any absorption (or reflection or scattering) of light by the cuvette and other species in the reference solution.

### GENESYS™ 20 Spectrophotometer

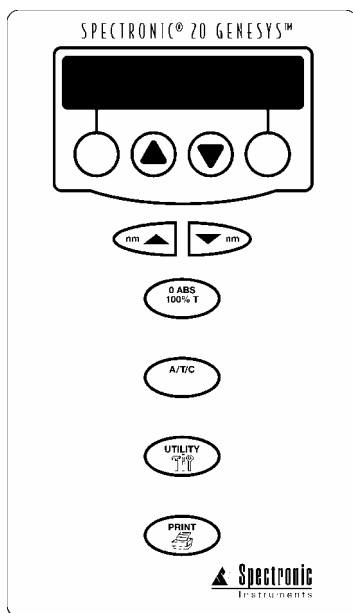
1. On / Off switch
2. LCD display
3. Sample compartment hood
4. Keyboard
5. ----
6. Lamp compartment door



The power switch is located on the back of the instrument. When you turn on your GENESYS™ 20 spectrophotometer, the power-on

sequence. This sequence includes checking the software revision, initializing the filter wheel and the monochromator. The power-up sequence takes about two minutes to complete. *Allow the instrument to warm up for 30 minutes before using it.*

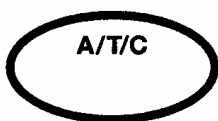
### Keyboard Layout of GENESYS™ 20 spectrophotometer



### Absorbance and % Transmittance Measurements

Note: Be sure the cell holder is empty before turning on the instrument.

On and off switch location



**1**

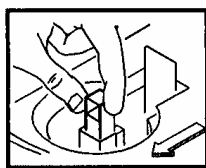
Press **A/T/C** to select the absorbance or % transmittance mode. The current mode appears on the display.



**2**

Press **nm ▲** or **nm ▼** to select the wavelength.

**Note:** *Holding either key will cause the wavelength to change more quickly.*



**3**

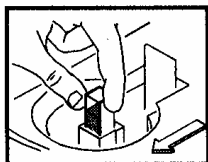
Insert your blank into the cell holder and close the sample door.

**Note:** *Position the cell so the light (indicated by the arrow in drawing) passes through the clear walls.*



**4**

Press **0 ABS/100%T** to set the blank to 0 A or 100%T.



**5**

Remove your blank and insert your sample into the cell holder. The sample measurement appears on the LCD display.

### **Error Messages**

This section lists the messages generated to alert you of errors or other abnormal conditions. The instrument recognizes two types of errors. With the first type, the instrument is still functional; with the second, the instrument is not functional until the condition is resolved.

#### **Flashing Data Display**

This condition indicates that the sample has an absorbance or a transmittance value below or above the photometric range of the instrument. The display flashes until the condition is resolved.

#### **Sample too dark**

This condition indicates that the instrument has been asked to zero a sample with a high absorbance at a low energy point. The instrument beeps three times to announce the message, the message remains on the display for two seconds, then the normal display returns.

#### **Sample too bright**

This condition indicates that the instrument has been asked to zero a sample while the door of the sample compartment is open. The instrument beeps three times to announce the message, the message remains on the display for two seconds, then the normal display returns.

### **Determining the Blank Cuvette**

1. Obtain a pair of cuvettes. One will be used for the blank solution and the other will be used for the standard and unknown samples.
2. Fill both cuvettes with the solvent and wipe the outside with a Kimwipe to make sure it is clean and dry (no fingerprints!). Be sure to always add enough solution to reach the to within 1/8 of the triangular mark on the cuvette. This will insure that all incident radiation passes through the solution. It is also critical that the

triangular mark on the cuvette be facing you when inserted in the sample holder for all measurements.

3. Insert one filled cuvette into the sample holder that is located under the hood to the left. Close the hood.
4. Calibrate to 0 absorbance by pressing the 0 ABS/100%T key. Remove the cuvette, insert the other cuvette, and read the absorbance for this second cuvette. If the reading is less than 0 absorbance, then this second cuvette will be used as the blank cuvette; if the reading is greater than 0 absorbance, then the first cuvette will be used as the blank cuvette.

### **Calibrating the Instrument**

The calibration procedure must be performed for absorbance measurements taken at each different wavelength.

1. Select the desired wavelength by pressing the up or down “wavelength selector” arrow keys of the key pad of the instrument.
2. Insert the “blank” cuvette containing the blank reference solution (which is acidic solvent for this experiment) into the sample holder and close the hood.
3. Calibrate to 0 absorbance by pressing the 0 ABS/100%T key.
4. Remove the “blank” cuvette. Do not re-adjust any dials at this point. You are now ready to insert the other cuvette containing the sample of choice for a measurement at this particular wavelength.
5. Repeat steps 1-4 for all measurements that take place at different wavelengths.

Question A: Why do we allow electronic instruments to warm-up before use?

Question B: Why is it important not to have fingerprints on the cuvette?

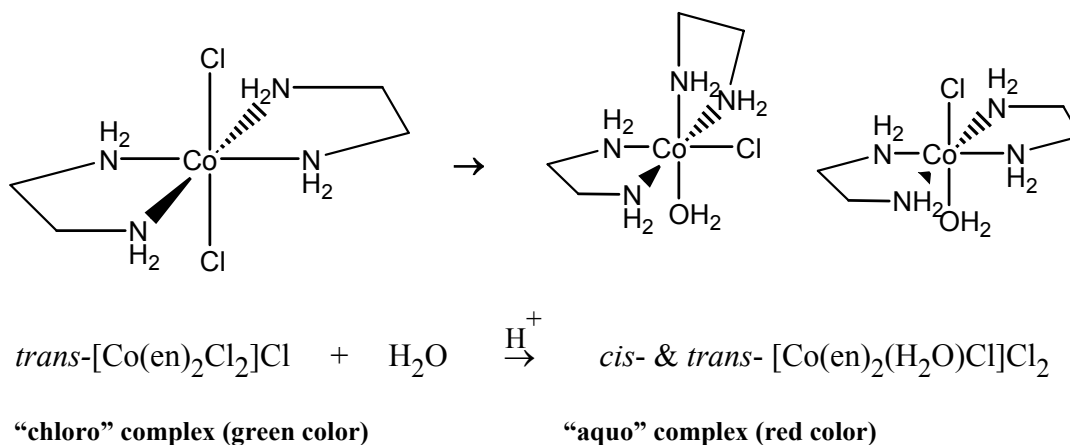
Question C: Why is it important to align the cuvette in the sample holder the same way each time?

## Introduction

In the present exercise you will, first, experimentally determine the wavelengths of the visible absorption maxima of two noninteracting compounds and measure the molar absorptivity for both of them at these two different wavelengths. Then, you will measure the absorption of an unknown mixture of two compounds in order to determine the concentration of each species.

The two compounds referred to above are the green “chloro” complex synthesized in the last experiment and a red “aquo” derivative, which you will make from the solution of the green complex.

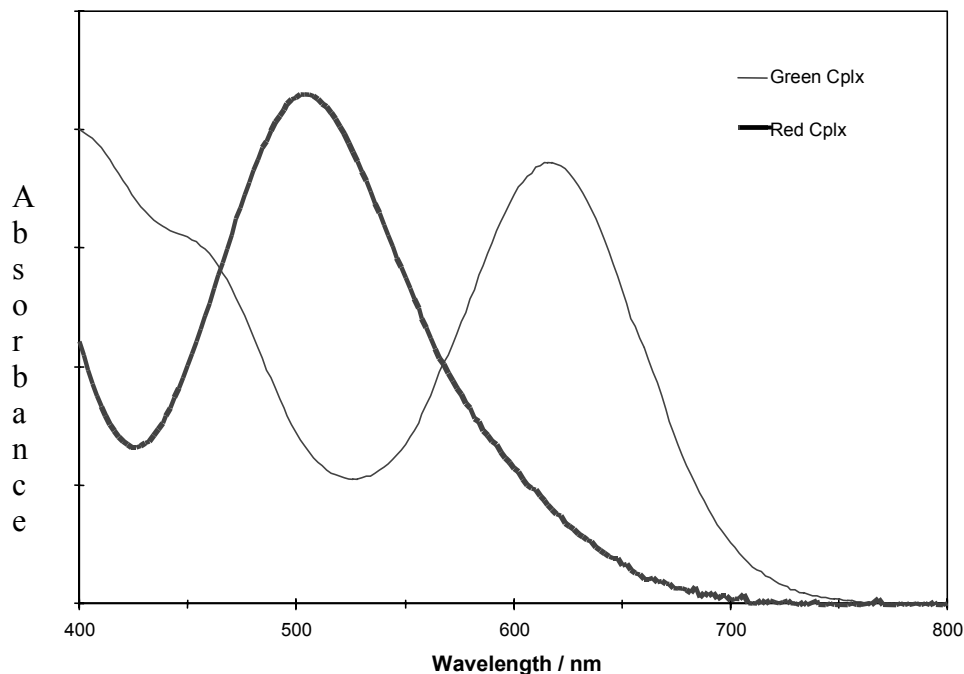
In acidic solutions with low free chloride ion concentrations, one of the chloride ions in the *trans*-[Co(en)<sub>2</sub>Cl<sub>2</sub>]<sup>+</sup> complex ion can be replaced by a water molecule to give an equilibrium mixture of the *cis* and *trans* isomers of the aquo complex, [Co(en)<sub>2</sub>(H<sub>2</sub>O)Cl]<sup>2+</sup>. This reaction is accompanied by a color change from green to red:



Throughout this experiment, the green-colored *trans*-[Co(en)<sub>2</sub>Cl<sub>2</sub>]Cl will be referred to as the chloro complex, while the red-colored mixture of the *cis* and *trans* isomers of the hydrolysis product, [Co(en)<sub>2</sub>(H<sub>2</sub>O)Cl]Cl<sub>2</sub>, will be simply referred to as the aquo complex.

In the first part of this experiment you will determine the wavelength of maximum absorbance ( $\lambda_{\max}$ ) for the two complexes. Then, you will prepare four solutions of each complex of known concentrations. You will then measure the absorbance of each solution using a GENESYS<sup>TM</sup> 20 spectrophotometer at the wavelength corresponding to the maximum absorbance for the chloro complex and will graph the data, with concentration on the x-axis and absorbance on the y-axis, to form a Beer's Law plot. By finding the slope and knowing the path length of your cuvette cell, you will be able to calculate the molar absorptivity coefficient at that wavelength for that complex. You will repeat this for the second wavelength that corresponds to the maximum absorbance position for the aquo complex. These measurements (i.e. at both

wavelengths) will be carried out for solutions of chloro and aquo complexes. In the final portion of this experiment you will analyze solutions that contain a mixture of the two complexes. You will be able to determine the concentrations of both complexes in the mixture using your spectrophotometric measurements.



**Figure 4. Absorption Spectra of Cobalt(III) Ethylenediamine Complexes**

**Safety:** Treat the GENESYS™ 20 spectrophotometers with great care as they are expensive and delicate instruments. Wear your goggles.

**You will work in pairs, sharing equipment, labor, and experimental results.** The actual data analyses and the written reports must be done entirely independently of your lab partner or other students. Make sure that you avoid unauthorized collaboration and plagiarism. All suspected violations of the Code of Academic Conduct will be referred to Student Judicial Affairs

## **Experimental Procedure**

### **Part I. Solution Preparation and Aquation of the Chloro Complex.**

1. Prepare the solvent to be used in the spectrophotometric part of this experiment by adding 6 mL of 3 M sulfuric acid to 75 mL of water.
2. Use a 50 mL buret to dispense a measured  $35 \pm 0.2$  mL of this solvent into a clean, dry 50 mL Erlenmeyer flask. Place this 50 mL flask to above the liquid level in a 250 mL beaker containing an ice bath made roughly of equal parts of water and ice.

3. Accurately weigh ca.  $200 \pm 4$  mg sample of the green chloro complex and transfer it carefully to the flask of cooled, acidic solvent. Remove the flask from the cooling bath, swirl it several times to insure that the solid has completely dissolved and the solution is homogeneous, and then quickly fill another 50 mL buret (or a 25 mL buret) with this cold solution. Return the flask containing the rest of the green solution to the ice bath.
4. Dispense a measured  $17.5 \pm 0.2$  mL portion of the cold green solution into a second clean, dry 50 mL Erlenmeyer flask and then quickly return the green solution remaining in the buret to the still-cooling flask from which it originally came. At this point you should have two essentially identical 17.5 mL samples of the green solution in two different 50 mL flasks--one of them in an ice bath and the other one out on the bench top warming to room temperature.
5. Put the "warmer" flask into a 250 mL beaker that contains about 100 mL of boiling water for about 10 minutes, which should cause the green starting material to be converted entirely to the red aquated product. Cool the flask containing the red solution to room temperature. If you wish you can use an ice bath to speed up the cooling.

## **Part II. Spectral Assignments.**

Overview: Figure 4 contains the absorption spectra of the red and green complexes. You will need to determine the true absorption maxima of the two complexes. The instrument you will use to make this determination is the manually operated GENESYS<sup>TM</sup> 20 spectrophotometer.

1. You should start with the red solution. The red solution is stable so it is clearly the one to use to familiarize yourself with the instrument and experimental procedure.
2. Your *red* stock solution is a little too concentrated to give good results. Therefore, you will want to use a disposable pipet to put about 2 mL of it in a test tube and then dilute that with about 2 mL of the acidic solvent dispensed from the 50 mL buret.
3. Obtain a pair of cuvettes. One will be used for the "blank" solution and the other will be used for the standard and unknown samples. Determine which cuvette will be used for the "blank" solution by following the procedures given above under the section, "Determining the Blank Cuvette."
4. Fill the cuvette selected for the blank to within 1/8 of the triangular mark on the cuvette with the acidic solvent dispensed from the 50 mL buret.
5. Rinse the other cuvette with a small portion of the diluted *red* solution (aquo product) and then fill it to within 1/8 of the triangular mark on the cuvette with this solution.
6. From the scanned spectra given in Figure 4 shown above, estimate the wavelength of the absorption maximum of the *red* colored complex.

7. Select a wavelength that is about 10 nm lower than the estimated absorption maximum determined in step 6. Calibrate the instrument following the procedure given above under the section, "Calibrating the Instrument." Once you have established 0 absorbance using your blank solution, insert the sample cuvette and read the absorbance. Record your measurement.
8. Remove the sample cuvette, increase the wavelength by two nanometers (nm), and recalibrate the instrument using the blank cuvette. Re-insert the sample cuvette and read the absorbance. Record your measurement.
9. Repeat step 8 until you have taken absorbance measurements every two nanometers from 10 nm below your estimate to 10 nm above your estimate. This can be done fairly quickly if you team up, with one person recalibrating, measuring, and calling out the resulting absorbances and the other one recording the data. Switch roles half way through the process so each partner has an opportunity to perform both tasks.
10. Empty the sample cuvette. Rinse it with deionized water, and carefully blot it dry using a Kimwipe.
11. Repeat steps 2 and 5-10 above for the *green* solution (chloro complex) instead of the *red* and thereby find the true wavelength of the *green* complex absorption maximum. You need to work reasonably efficiently because when the sample of interest is the green solution at around room temperature it will be changing slowly with time. For step 2, mix 2 mL of the cold stock solution with 2 mL of solvent at room temperature and use the resulting solution *immediately* unless water condenses on the outside of the test tube in which you did the mixing. If that happens, hold the test tube tightly in your hand until it warms up to above the dew point. The absorbance measurement will be greatly affected if condensation occurs on the outside of the cuvette. For step 7, be sure to use the estimated wavelength of maximum absorption for the GREEN solution.
12. Review your absorbance readings for the two complexes. Determine the wavelength where maximum absorbance occurred for each colored complex. Record these wavelengths for use in Part III.

### **Part III. Collecting the Data Needed to Construct Beer's Law Plots.**

Overview: In this part of the experiment you will prepare eight solutions with known concentrations--four each of both the red and green solutes--and measure their absorbances at the wavelengths of the two absorption maxima you determined in Part II above; i.e., you will make a total of sixteen spectroscopic measurements.

1. Fill one 50 mL buret with the cold, green chloro solution and another one with the solution of its red aquation product. Evenly splitting up the workload within the partnership, dispense four measured  $3.0 \pm 0.2$  mL portions of each of these solutions into separate clean, dry test tubes. This entails numerous buret readings, but in order to maintain a reasonable degree of precision here you really do have to make them all. As always, immediately store the "green" tubes in the ice/water bath.
2. To the four "red" test tubes in turn use the 50 mL buret to add measured volumes of the acidic solvent as follows:  $2.0 \pm 0.2$ ;  $3.0 \pm 0.2$ ;  $4.0 \pm 0.2$  and  $5.0 \pm 0.2$  mL. (You should now have four tubes containing accurately measured total volumes of about 5, 6, 7, and 8 mL.)
3. With the proper technique measure the absorbance of each of these solutions at the wavelengths you determined above for the absorption maxima of both the red and the green complexes (for a total of 8 readings).
4. One test tube at a time, repeat steps 2 and 3 with the four samples of the green complex. As before, you can use the diluted samples immediately unless water vapor condenses on the outside of the test tube after mixing--in which case warm it up with your hand.
5. When you are finished with Part III, you should have collected a total of 16 different absorbance readings here; 8 for the red solutions and 8 for the green solutions.

### **Part IV. Collecting Data for the Analysis of a Mixture of the Two Complexes.**

1. Your TA will give you a solution that contains a mixture of the red and green complexes prepared by allowing a solution of the chloro complex partially to convert to the aquo product. Measure the absorbance of the mixture at the wavelength of maximum absorbance found for green solution in Part I and measure the absorbance of the mixture at the wavelength of maximum absorbance found for the red solution in Part I. (You should have a total of 2 readings.)

<b>WASTE:</b> The contents of the test tubes must be discarded to the waste container labeled <b>Cobalt waste.</b>
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**Data Analysis – Post-laboratory exercises will guide you through these calculations.**

### **Part III. Making the Beer's Law Plots.**

Overview and a Modest Theoretical Excursion: In this part of the experiment you will manipulate the data collected in Part III to obtain the values of *effective molar absorptivity*, symbolized by  $\epsilon^*$ , for both the red and the green complexes at the two wavelengths of interest here.

Beer's Law in the form in which you have seen it previously,  $A = \epsilon\lambda C$ , strictly applies only to a slender beam of monochromatic light passing through a cell with flat, parallel windows. In the present experiment the light beam is not small and the cell is not a rectangular parallelepiped, so the path length,  $\lambda$ , cannot be defined.

Operationally, the detector outputs a signal that is the average of the exit intensity over the cross section of the cylindrical cuvette. If necessary one could measure what might be called an "effective path length" for each cuvette by measuring the absorbance of a solution of some absorber whose true molar absorptivity has already been measured, but we do not have to do this here. Instead, we can combine the molecular property  $\epsilon$  and the effective path length of the cell being used for all the measurements into a new constant,  $\epsilon^*$ , whose magnitude is valid only for the particular system (absorber and cuvette) under study. Beer's Law then takes the simpler form  $A = \epsilon^*C$ . (Note that the way it is defined here  $\epsilon^*$  must have dimensions  $M^{-1}$  because absorbance is dimensionless.)

We shall henceforth distinguish the four different  $\epsilon^*$  values by two-letter identifying subscripts: namely, GG, GR, RG and RR. The meanings of these combinations are as follows:

GG = Green solution examined at the wavelength of the Green absorption maximum;  
GR = Green solution examined at the wavelength of the Red absorption maximum;  
RG = Red solution examined at the wavelength of the Green absorption maximum;  
and RR = Red solution examined at the wavelength of the Red absorption maximum.

According to the modified form of Beer's Law introduced above, one can determine the numerical value of  $\epsilon^*$  ( $= \epsilon\lambda$ ) for some solute at a given wavelength by determining the slope of a plot of absorbance vs. concentration.

You may find it useful to use a spreadsheet program to do the following calculations.

1. Calculate the molar concentrations of the eight solutions you studied.
2. Calculate the 16 absorbances and use them along with the concentrations calculated in Step 1 to prepare four graphs of absorbance vs. concentration. Each of your plots should occupy most of an 8.5"x11" sheet of graph paper. From these plots determine the four pertinent values of  $\epsilon^*$ . (You must plot the data even if you have a hand

calculator that will do linear regression for you. Many scientists have been blindsided by doing computer fits to data that really did not obey the fitting equation. If your plots are nicely linear then you can certainly get the slope the easy way.)

#### **Part IV. Spectrophotometric Analysis of a Mixture of the Two Complexes.**

Theory: The absorbance of a mixture of absorbing species that do not interact with each other in any way is simply the sum of the absorbances of the individual species. This means that in a solution that contains both the red and the green complexes in significant amounts the absorbance at  $\lambda_{\max}$  of the green form will be given by the equation

$$A^G = \epsilon^*_{GG} [C_G] + \epsilon^*_{RG} [C_R]$$

and the absorbance at  $\lambda_{\max}$  of the red form will be given by

$$A^R = \epsilon^*_{GR} [C_G] + \epsilon^*_{RR} [C_R]$$

where  $[C_X]$  is the molar concentration of  $X = R, G$ .

It follows from this that if the four  $\epsilon^*$  values are all known, the individual concentrations in a mixture of the two species can be obtained by measuring its absorbance at the two maximal wavelengths and solving the pair of simultaneous equations given just above. (Students who know some linear algebra will recognize that these equations can be compacted into the matrix equation.)

Calculate the concentrations of the green “chloro” complex and the red “aquo” complex in the mixture.

### Post-laboratory Exercise Questions

The following series of questions pertains to the analysis of the data in Parts III and IV. Your data and resulting calculations will be verified as you proceed through the exercise. You should have worked through the analysis of Parts III and IV in your notebook before beginning; otherwise, you may need an hour or so to complete this exercise. You may leave the exercise at any point and continue it later.

Why do we allow electronic instruments to warm-up before use?

Why is it important not to have fingerprints on the cuvette?

Why is it important to align the cuvette in the sample holder the same way each time?

In part I step 3, you were instructed to weigh approximately 200 mg of the green chloro complex. Please enter the precise mass **in milligrams** of the green chloro complex used to prepare the solution.

In part I step 2, you were instructed to dissolve the green chloro complex into approximately 35 mL of acidic solvent. Please enter the precise volume in milliliters of the acidic solvent used to prepare the solution (e.g. 35.02). Your precision should be to a hundredth of a milliliter.

If you weighed out 203 mg of the green chloro complex and dissolved it in 35.14 mL of acidic solvent, the molarity of your stock solution would be 0.0202 M. Using your precise value of mass and volume that you entered above, please enter your calculated value for the concentration of the original green chloro complex stock solution in moles per liter.

Please enter the value of the wavelength **in nanometers** of the maximum absorbance for the **GREEN** solution.

Please enter the value of the wavelength **in nanometers** of the maximum absorbance for the **RED** solution.

The original red stock solution has the same concentration as the green stock solution since one mole of the red aquo complex forms for each mole of the green chloro complex that is converted. Therefore, the concentrations of the two sets of four diluted solutions, the red and green, are equal for each respective concentration. Enter the four calculated values of the concentration of the solutions you prepared. Enter them in decreasing order (highest to lowest) of concentration in units of moles per liter.

(The order of entry is very important here.)

Next, we will collect your data for the absorbance of each of your diluted samples for both the green and red solutions that is used subsequently to verify your Beer's law plots. We will refer to your Beer's law data using the shorthand suggested in your laboratory manual. GG, GR will be the measurements of the green solution at the maxima of the green and red complexes respectively and RG, RR will be the measurements on the red solution at the maxima of the green and red complexes respectively. It is important to correspond the absorbance readings with the correct diluted sample. To do this you will need to enter, first, the absorbance that corresponds to the diluted sample of entry #1 above; second, the absorbance corresponding to diluted sample of entry #2; etc... This means that your absorbance values will be in decreasing order. Please check for this carefully as it will affect the slope calculations.

**First, let's collect the data needed for the determination of the GG effective extinction coefficient.** The absorbance values should have a value between 0 and 1. Please enter the 4 values of the GG absorbance measurement in corresponding order of solution concentration (high concentration to low concentration; values of absorbance will be running in the same sense, high absorbance to low absorbance and expressed to 3 significant digits).

Plot the values of GG Absorbance versus Concentration on a spreadsheet or a programmable calculator, and carefully determine the slope of the best straight line fit through the data. The slope values for GG, GR, RG, and RR must be accurately determined because they will enable you to solve for the unknown concentration of your mixture of the two cobalt complexes. [CLICK HERE](#) (excel tutorial available on-line) if you would like assistance using an Excel spreadsheet for determining the slope of a best fit line. Enter the value of the slope of A vs. C. This is your effective extinction coefficient for GG.

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**Now let's collect the data needed for the determination of the GR effective extinction coefficient.** The absorbance values should have a value between 0 and 1. Please enter the 4 values of the GR absorbance measurement in corresponding order of solution concentration (high concentration to low concentration; values of absorbance will be running in the same sense, high absorbance to low absorbance and expressed to 3 significant digits).

Plot the values of GR Absorbance versus concentration on a spreadsheet or a programmable calculator, and carefully determine the slope of the best straight line fit through the data. The slope values for GG, GR, RG, and RR must be accurately determined because they will enable you to solve for the unknown concentration of your mixture of the two cobalt complexes. [CLICK HERE](#)(excel tutorial available on-line) if you would like assistance using an Excel spreadsheet for determining the slope of a best fit line. Enter the value of the slope of A vs. C. This is your effective extinction coefficient for GR.

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**Now let's collect the data needed for the determination of the RG effective extinction coefficient.** The absorbance values should have a value between 0 and 1. Please enter the 4 values of the RG absorbance measurement in corresponding order of solution concentration (high concentration to low concentration; values of absorbance will be running in the same sense, high absorbance to low absorbance and expressed to 3 significant digits).

Plot the values of RG Absorbance versus concentration on a spreadsheet or a programmable calculator, and carefully determine the slope of the best straight line fit through the data. The slope values for GG, GR, RG, and RR must be accurately determined because they will enable you to solve for the unknown concentration of your mixture of the two cobalt complexes. [CLICK HERE](#) (excel tutorial available on-line) if you would like assistance using an Excel spreadsheet for determining the slope of a best fit line. Enter the value of the slope of A vs.C. This is your effective extinction coefficient for RG.

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**Now let's collect the data needed for the determination of the RR effective extinction coefficient.** The absorbance values should have a value between 0 and 1. Please enter the 4 values of the RR absorbance measurement in corresponding order of solution concentration (high concentration to low concentration; values of absorbance will be running in the same sense, high absorbance to low absorbance and expressed to 3 significant digits).

Plot the values of RR Absorbance versus concentration on a spreadsheet or a programmable calculator, and carefully determine the slope of the best straight line fit through the data. The slope values for GG, GR, RG, and RR must be accurately determined because they will enable you to solve for the unknown concentration of your mixture of the two cobalt complexes. [CLICK HERE](#)(excel tutorial available on-line) if you would like assistance using an Excel spreadsheet for determining the slope of a best fit line. Enter the value of the slope of A vs.C. This is your effective extinction coefficient for RR.

**We are now ready to complete the analysis of Part IV data.** Using the values of the effective extinction coefficients you determined from your slopes and the values of the absorption you obtained for the mixture at the spectral maxima for the green and red solutions, solve the 2x2 simultaneous linear equations for the concentrations of the green and red complexes in the mixture. When you have an answer for these concentrations, we will use the same data and verify your result.

The absorbance values should have a value between 0 and 1. Please enter the absorbance you measured for the mixture of cobalt complexes at the wavelength of absorption maximum for the **GREEN** complex.

The absorbance values should have a value between 0 and 1. Please enter the absorbance you measured for the mixture of cobalt complexes at the wavelength of absorption maximum for the **RED** complex.

Please enter the values you calculated for the concentrations of the green and red complexes in the mixture. (Use 3 significant figures.)

