

Experiment 17

Beer's Law

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Name:

Date:

Key Objectives

1. Terminology - wavelength, absorption, and transmission.
2. Dilution and use of $M_1V_1 = M_2V_2$.
3. Creation and Use of Calibration curves.
4. Use of linear equation - $y = mx + b$.
5. Absorption and Emission of light from an atom or molecule.

Discussion

The use of spectroscopy to study light and matter has a long history. This lab will examine the use of a spectrometer to measure the absorptions spectra of a compound, and using Beer's Law to determine the concentration of an unknown solution. (Unfortunately this lab is not about brewing, drinking, or really anything to do with beer.)

Light and Matter

Every substance has an infinite number of possible electron configurations. The configuration that corresponds to the lowest energy level of a substance is called its ground state. Energy can be absorbed by a substance to raise the electron from the ground state to a higher energy state/higher orbital or (excited state) as shown in Figure 17.1a. The absorbed energy can be emitted as light when an electron in a higher energy level falls to a lower energy level emitting a photon of light as shown in Figure 17.1b.

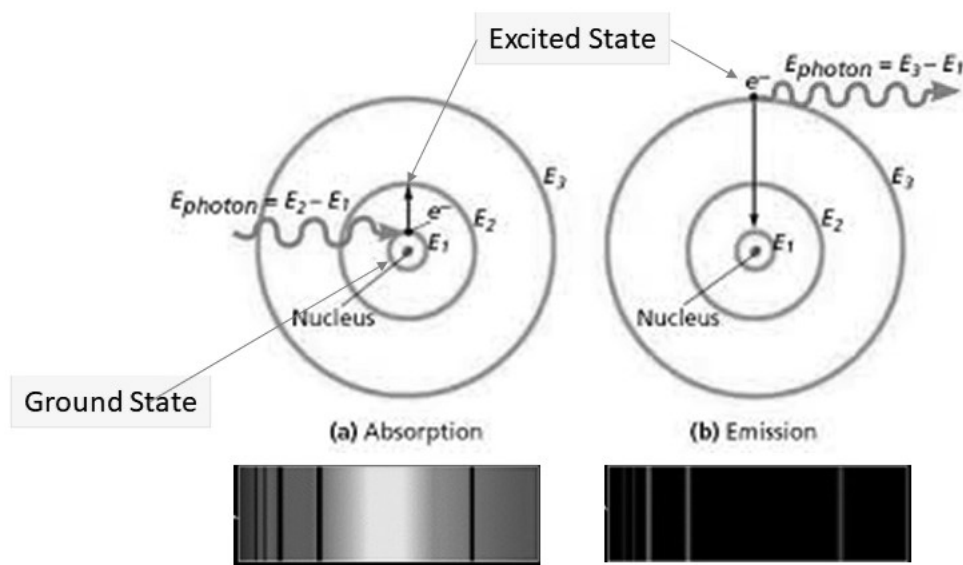


Figure 17.1: (a) Absorption spectra and (b) Emission spectra credit: unknown

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Planck's Law shows that a substance will absorb/emit light according to the equations shown in Figure 17.2.

$$\begin{aligned} \Delta E &= h\nu & h &= \text{Planck's Constant} = 6.626 \times 10^{-34} \text{ J}\cdot\text{s} \\ \Delta E &= \frac{hc}{\lambda} & c &= \text{speed of light} = 3.0 \times 10^8 \text{ m/s} \\ & & \nu &= \text{frequency (Hz or 1/s)} \\ & & \lambda &= \text{wavelength (m or nm)} \end{aligned}$$

Figure 17.2: Planck's Law and Absorption and Transmission of Light.

The spectrum of radiation is vast and how each type of radiation interacts with matter is different. In this experiment we focus on the visible portion of the spectrum corresponds to wavelengths between 400 and 700 nm as shown in Figure 17.3a. When light is incident on an object the object can absorb light whose wavelength obeys Planck's Law, all other wavelengths are transmitted or reflected. Our perception of color is then due to the complementary colors (Figure 17.3b) in the transmitted or reflected light. For example, if an object absorbs light near 600 nm (orange) appears blue while an object that absorbs light near 490 nm (blue-green) appears red. A colorless object does not absorb any light, thus appearing white. An example is shown in Figure 17.4b, a solution of NiSO_4 appears blue-green because all other colors are absorbed.

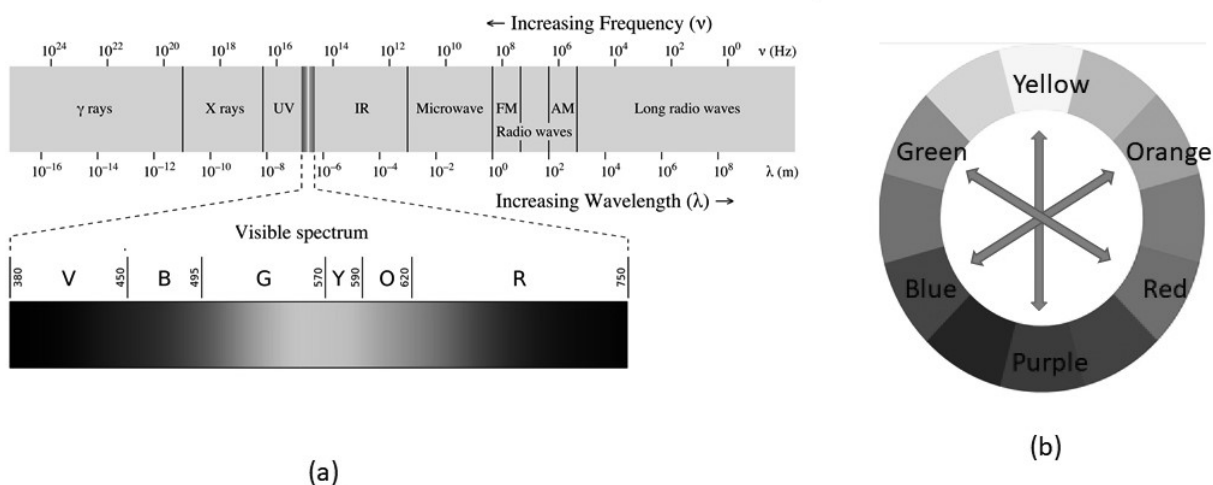


Figure 17.3: (a) Wavelength of different types of radiation highlighting the visible spectrum. (b) Complementary color wheel. credit: (a) https://commons.wikimedia.org/wiki/File:EM_spectrumrevised.png (b) https://simple.wikipedia.org/wiki/Complementary_color

Spectrometers

If we consider light of only one wavelength being transmitted through a solution we can define two quantities. Transmittance (T) is the ratio of the initial intensity of light, I_0 and the light that leaves the solution as I. Absorbance (A) is a related quantity defined as the negative log of the transmittance.

$$\begin{aligned} \text{Transmittance (T)} & T = \frac{I}{I_0} \\ \text{Absorbance (A)} & A = -\log T \end{aligned}$$

The device used to measure Transmittance and Absorbance is called a spectrometer. The device func-

tions by splitting light into its component wavelengths and measuring the intensity before and after it passes through a solution. Spectrophotometers come in a variety of shapes, sizes, and most importantly for us price.

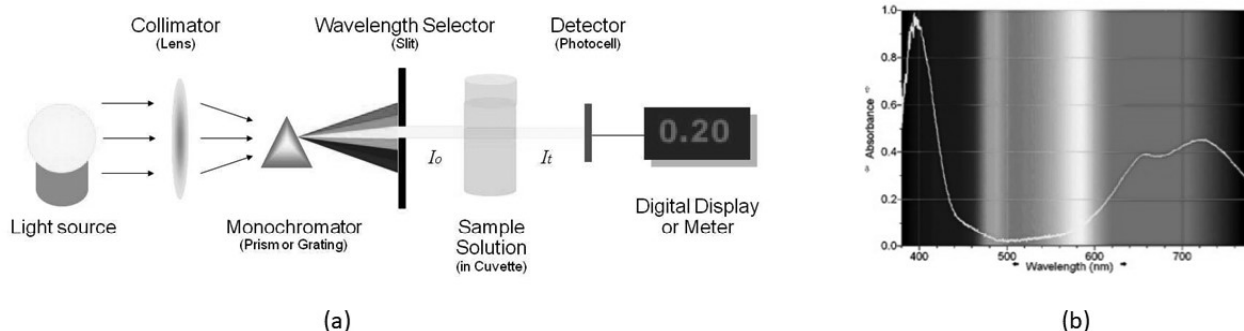


Figure 17.4: (a) Schematic of a spectrophotometer. (b) Absorption spectrum of NiSO_4 . credit: (a) http://chemwiki.ucdavis.edu/Physical_Chemistry/Kinetics/Reaction_Rates/Experimental_Determination_of_Kinetics/Spectrophotometry (b) <https://sites.google.com/site/frinanostream/tutorials/spectrophotometry>

Absorption Spectrum

Given a colored substance dissolved in a colorless liquid (water), the colored substance will absorb different wavelengths of light. Using a spectrometer one can measure the Absorbance at many different wavelengths to produce an Absorption Spectrum. Figure 17.5 shows data and a typical absorption spectra. Note the Absorption Maximum marked in the data and on the graph. This is the most sensitive part of the spectrum to changes in concentration. In the lab you will measure the spectrum of your compound and determine the Absorption Maximum for it.

For a series of solutions (discussed below) we see that the Absorption Maximum decreases as the concentration of the solution decreases as shown in Figure 17.6 below.

Dilutions

Dilutions can best be thought of as taking a concentrated solution and making a less concentrated solution from it. Similar to the way that one can take frozen OJ (the juices, not the football player) and add water to it. When making concentration measurements, it is often necessary to make a series of solutions of different concentrations to make a calibration curve. This process is best accomplished by taking a solution of known concentration and performing a series of dilutions to make less concentrated solutions. The following formula is most often used:

$$M_1 V_1 = M_2 V_2$$

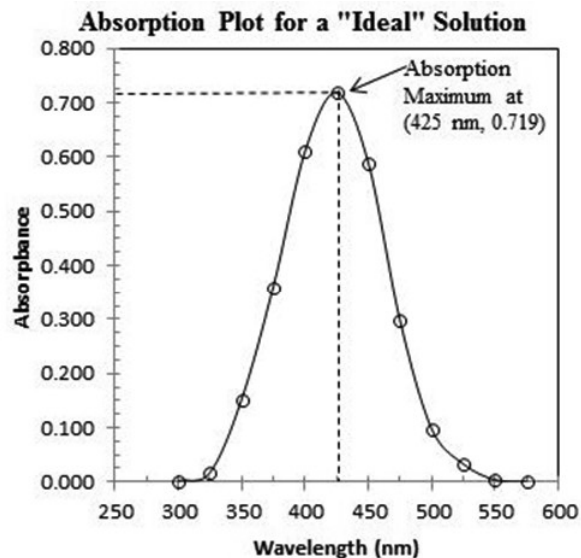
M_1 = Molarity of Initial Solution
 V_1 = Volume of Initial Solution
 M_2 = Molarity of Final Solution
 V_2 = Volume of Final Solution

Note that V_2 is the final volume of the solution, for example to calculate the Molarity of Solution 2 in the table below, you take 4 mL of the concentrated solution, and add 1 mL of water, the final volume (V_2) would be 5 mL.

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Wavelength (λ) (nm)	Absorbance (A)
300	0.002
325	0.017
350	0.152
375	0.360
400	0.610
425	0.719
450	0.589
475	0.297
500	0.097
525	0.033
550	0.004
575	0.001

(a)



(b)

Figure 17.5: (a) Raw data for an absorption spectrum as collected in lab. (b) Plot of the data showing the absorption maximum. Note that the data is made up and much nicer looking than what will be collected in lab. credit: author

For Solution 2:

$$M_1 V_1 = M_2 V_2$$

$$(0.0120 \text{ M})(4.0 \text{ mL}) = M_2(5.0 \text{ mL})$$

$$M_2 = 0.00960 \text{ M}$$

$$M_1 = 0.0120 \text{ M}$$

$$V_1 = 4.0 \text{ mL}$$

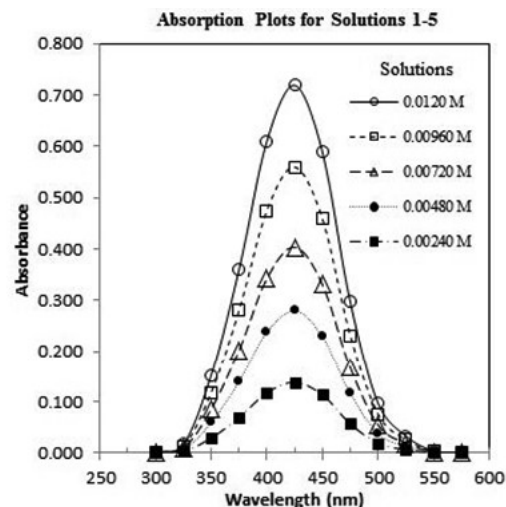
$$M_2 = \text{Molarity of Final Solution}$$

$$V_2 = 5.0 \text{ mL}$$

The table in Figure 17.6 summarizes a series of 4 dilutions. Figure 17.6 also shows the absorption spectra for five solutions, the original, and four dilutions. Note that the Absorption Maximum decreases as the concentration of the solutions decreases.

Solution Number	M_1 (M)	V_1 (mL)	M_2 (M)	V_2 (mL)	Final Conc. (M)
1	0.0120	-	-	-	-
2	0.0120	4	x	5	0.00960
3	0.0120	3	x	5	0.00480
4	0.0120	2	x	5	0.00280
5	0.0120	1	x	5	0.00240

(a)



(b)

Figure 17.6: (a) Concentration of 5 solutions after dilution using $M_1V_1 = M_2V_2$. (b) Absorption data for solutions of different concentration. concentration vs absorbance. Note that the data is made up and much nicer looking than what will be collected in lab. credit: author

Beer's Law

Beer's Law (not the one you are probably thinking of) states that the absorbance at a given wavelength is directly proportional to the molar concentration of the substance. The proportionality constant is often termed the molar absorptivity constant. Note that because absorbance is a unitless, the units for the proportionality constant (k) are simply the inverse of normal concentration units. The constant is specific to each substance and specific to a given wavelength.

$$A = kM$$

A = Absorbance
 k = molar absorptivity constant (L/mol or M^{-1})
 M = Molarity of the solution (mol/L or M)

Note that the equation for Beer's Law has the same form as that of a straight line. Given a series of solutions with known concentration plotting Concentration vs. Absorbance should yield a straight line whose slope is the molar absorptivity, and the equation of the line allows calculation of the concentration of an unknown concentration.

$$y = mx + b$$

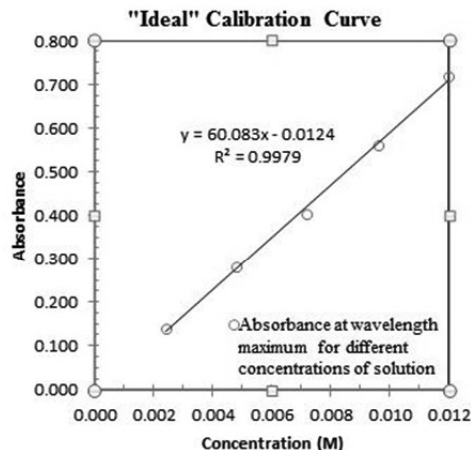
y = Absorbance
 m = molar absorptivity constant (L/mol or M^{-1})
 x = Molarity of the solution (mol/L or M)

Figure 17.7 shows the relationship between concentration of solution and absorption of the solution. Note that it is linear and the curve can be used to determine the concentration of an unknown solution using the equation of the line ($y = mx + b$).

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Solution Number	Concentration (M)	Absorbance (at 425 nm)
1	0.0120	0.719
2	0.00960	0.560
3	0.00720	0.404
4	0.00480	0.280
5	0.00240	0.138

(a)



(b)

Figure 17.7: (a) Concentration vs Absorbance data using data and graph from Figure 17.6. (b) Resulting curve showing the relationship between concentration and measured absorbance. The curve could be used to determine the concentration of an unknown solution. Note that the data is made up and much nicer looking than what will be collected in lab. credit: author

Hydrates

Certain ionic compounds form with large spaces in their crystalline structures as shown in Figure 17.8. These spaces are a perfect fit for many small molecules which are trapped there. When water is trapped in the lattice these compounds are often referred to as **hydrates**. The water which is trapped in the hydrate lattice is often called the **water of crystallization** or water of hydration. In a hydrate the water molecules are a different/distinct part of the compound and are not bonded to the crystalline lattice, and instead held in place by Intermolecular Forces (IMF's) that are significantly weaker than those forming the ionic lattice. To indicate this, the formula for hydrates is written with a dot used to separate the formula of the ionic salt from that of the trapped water molecules ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$). The name of the compounds are slightly different also, the ionic part is named as usual, and we indicate the additional water by giving the number of water molecules (using di, tri, etc.) and the word hydrate. For example $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ would be called calcium sulfate dihydrate.

The molecular weight calculation of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ is shown below.

$$\begin{aligned} 2 \text{ Ca: } & 1 \times 40.078 = 40.078 \\ 1 \text{ S: } & 1 \times 32.065 = 32.065 \\ 4 \text{ O: } & 4 \times 15.999 = 63.996 \\ 4 \text{ H: } & 4 \times 1.0079 = 4.0316 \\ 2 \text{ O: } & 2 \times 15.999 = 31.998 \\ \text{Molecular Weight} & = 172.169 \text{ g/mol} \end{aligned}$$

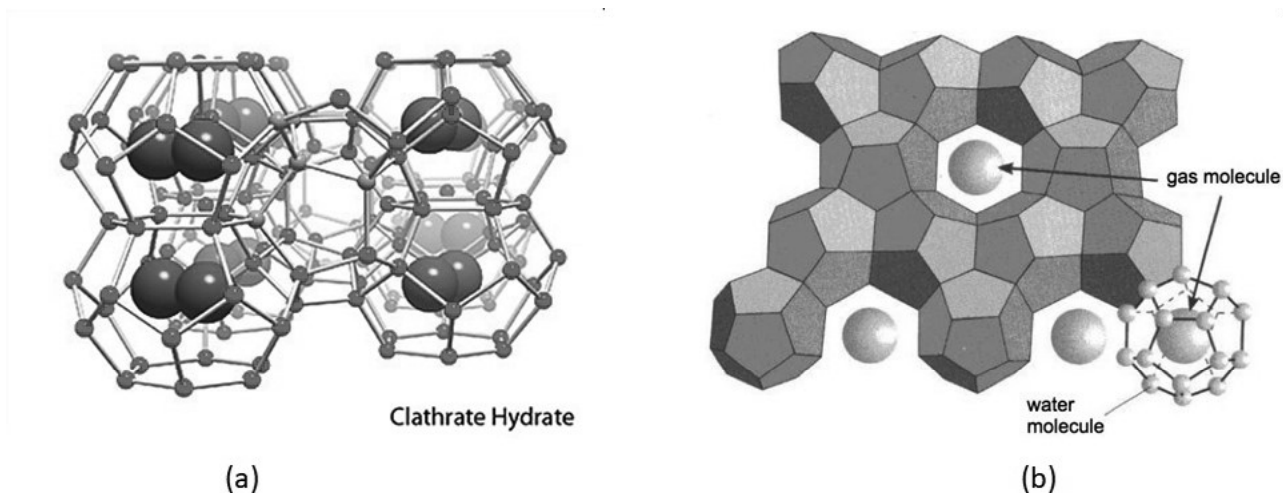


Figure 17.8: (a) and (b) Hydrates are crystalline compounds that have water trapped in the empty space within the crystalline lattice. Similar compounds shown here are clathrate hydrate structures which are crystalline compounds in gas molecules are trapped in the spaces inside the crystal structure of frozen water. credit: (a) <https://ps.uci.edu/group/kcjanda/research/gas-hydrate-structure> (b) http://dusk.geo.orst.edu/oceans/deep_currents.html

Procedure

You will prepare a solution of known concentration and perform a series of dilutions while measuring the absorption of each solution. You will then calculate a calibration curve and determine the value of the Absorptivity Constant. In the final part you will be given a solution of unknown concentration and must determine the concentration.

Your goal for Day 1 is to make your solutions and measure the absorption spectrum of each solution. **Before** Day 2, you must plot the data and determine the Molar Absorptivity constant. Failure to do so will result in a zero for the second part of the lab. During Day 2 you will be given an solution of Unknown concentration and must determine the concentration by performing a series of dilutions until the solutions Absorption Maximum lies on your calibration curve. You will then make a final measurement on a solution prepared in a single step.



Dispose of all mixtures in the appropriately labelled container in the hood.

Day 1 - Preparing the Initial Solution.

Using the volumetric glassware provided in class, prepare 100 mL of a 0.150 M solution of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$. Show work for any calculations performed on the Results page.

1. Calculate the Molecular Weight (MW) of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$. (show work)
2. Calculate the mass of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ required to make a 100.0 mL solution of 0.150 M $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$. (show work)
3. Depending on the sample given, it may be necessary to grind the large crystals in a mortar

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and pestle in order to get a sample suitable for measuring, and to insure the consistency of the sample.

4. Measure the required mass of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and add it to the 100 mL Volumetric Flask.
5. Add about 75 mL of water and swirl the solution until all the $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ is dissolved.
6. Finish the solution by adding water to the line. You may want to use a pipet for the last milliliter to maximize precision
7. When done with your solution for the day, store it in a properly labelled Erlenmyer flask and seal it with wax paper.

Day 1 - Preparing the Dilutions

1. Obtain 2 volumetric pipets, 10 test tubes and a test tube rack.
2. Rinse the volumetric pipet that will be used with the 0.150 M $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ several times, discarding the solution each time.
3. Using the table below prepare 10 test tubes by taking the required volumes of 0.150 M $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and water. Mix the solutions, do not use a stopper or your finger.
4. Calculate the molarity of each solution and enter the data in the Results section. In the space below the table in the Results section show work for Test Tubes 2 and 7.

Test Tube Number	mL of 0.150 M $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	mL of H_2O
1	10.0	0.0
2	9.0	1.0
3	8.0	2.0
4	7.0	3.0
5	6.0	4.0
6	5.0	5.0
7	4.0	6.0
8	3.0	7.0
9	2.0	8.0
10	1.0	9.0

Day 1 - Measuring the Absorption Spectra of Your Solutions

1. Before making any measurements, rinse the spectrometers sample cell with distilled water. Dry it completely with tissue paper. Rinse it with a small portion of your sample (discard the solution), then fill it with the sample to be tested.
2. For each test tube measure the absorption spectrum.
3. Save your data for use later.
4. Plot the entire spectrum of Test Tube 1 for your lab report (Figure 17.1). Determine the Absorbance Maximum. (Attach the spectrum to the end of the Results section.)
5. In the results section, list the value of the maximum in the Data Table 2.
6. Using the Absorbance Maximum, determine the Absorbance for each of the test tubes at the maximum and record the data in the Results section.
7. Make a calibration curve (Figure ??) for your sample and for the linear portion of the graph determine the equation of the line and the Molar Absorptivity constant. (Attach the calibration curve to the end of the Results section.)
8. Before you come to lab on day 2 be sure you have completed the previous section. If you have any bad data you will be allowed time to remeasure it.

Day 2 - Determining the Approximate Concentration of an Unknown Solution (Table 3)

In this section we will calculate an **approximate** concentration of your unknown solution.

1. Before you come to lab on day 2 be sure you have completed the previous section. If you have any bad data you will be allowed time to remeasure it.
2. Obtain an unknown from the instructor.
3. Plan ahead, you will only be given 15.0 mL of your Unknown and must make all measurements with that sample. If you plan poorly or make too many mistakes you will not be able to complete this section and will receive a zero.
4. Determine an approximate concentration of your unknown by diluting a small sample (5 mL) of your unknown with (5 mL) of water. Measure the absorbance.
5. Take 5 mL of the previous solution and add 5 mL of water and measure the absorption.
6. Repeat the previous step until the absorbance lies within the range of your calibration curve. Record your data. If you need more than 7 dilutions use additional paper to record your data (and your likely doing something wrong!)
7. Calculate the concentration of your **diluted** solution. (Show work.) (This is also the first question after the table.)
8. Calculate the concentration of your **undiluted** solution. (Show work.) (This is also the second

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question after the table.)

Day 2 - Determining the Actual Concentration of an Unknown Solution (Table 4)

The above measurements were an approximation and may contain a significant amount of error due to performing multiple dilutions. You will now calculate, prepare, and measure your unknown using a single dilution.

1. Calculate the required dilution so that by performing a single dilution your unknown will absorb on your calibration curve. (Show work.) Hint: Use ($M_1V_1 = M_2V_2$) and use the approximate value for M_1 , a value in the linear part of your calibration curve for M_2 and 1 or 2 mL for V_1 . This will yield a value for V_2 which can be rounded to the nearest mL to make measuring easier).
2. Perform the above dilution, and measure the absorption. If your absorption is on the calibration curve you are done, it does not, redo the calculation and/or dilution until it does. Record all trials (good or bad) in the Results section. (If you need to redo the final dilution more than 3 times record it on an additional piece of paper.)

Results

Data Table 1: Preparing the Initial Solution

Measurement	Value
Calculated MW of $\text{CoCl}_2 \cdot 6 \text{H}_2\text{O}$:	
Calculated mass of $\text{CoCl}_2 \cdot 6 \text{H}_2\text{O}$ required to make 100 mL of 0.150 M solution	
Actual Mass of $\text{CoCl}_2 \cdot 6 \text{H}_2\text{O}$ used:	
Actual Molarity of Solution:	

Table 17.1: Preparing the Initial Solution

Data Table 2: Concentration and Absorption Measurements

Attachments

1. Attach the absorption spectrum of test tube 1. Draw an arrow pointing to the Absorption Maximum, and label it. In the label include both the value of the wavelength and the value of the Absorption (Figure 17.1).
2. Attach the calibration curve and be sure to include the line fit (Figure ??).

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Test Tube #	Concentration	Absorption @ _____
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

Table 17.2: Concentration Calculations and Absorptivity Measurements

Post Lab Questions - Day 1

1. Why is it important to rinse the pipets and the spectrometer sample tube with your solution several times before making an actual measurement.

2. If you didn't do so in the Results section, show an example calculation for the concentration of Test Tube 2 and 7.

3. Graph the absorption spectra for test tube number 1 and attach it to the back of the lab. At what wavelength is the absorption maximum? What is the value of the absorption at the maximum?

4. Graph the calibration curve for test tube number 1-10 and attach it to the back of the lab. Write the equation of the line and the curve fit below. What is the value of the Absorptivity Constant (k) (i.e. the slope)?

5. What experimental errors could/did occur during the lab? Suggest a way to improve the lab (by avoiding or minimizing errors).

6. What color was your solution? Where did the Absorption Maximum occur? Does this make sense? Explain.

Data Table 3: Determining the Approximate Concentration of an Unknown

Series Dilutions for an Approximate Concentration				
Trial #	mL Solution	mL H ₂ O added	Absorption	Concentration (M)
	Unknown Solution	none		
1	5 mL Unknown	5 mL H ₂ O		
2	5 mL Solution 1	5 mL H ₂ O		
3	5 mL Solution 2	5 mL H ₂ O		
4	5 mL Solution 3	5 mL H ₂ O		
5				
6				
7				

Table 17.3: Approximate Concentration Calculations for the Unknown

- Using the last measured result in Data Table 3, use the calibration curve (the equation of the line, $y = mx + b$) to calculate the concentration of your diluted unknown. (show work).

- Using ($M_1V_1 = M_2V_2$) calculate the approximate concentration of your unknown.

- Using your Results from Data Table 3, calculate the values of V_1 and V_2 to use in order to perform a single step dilution to calculate the actual concentration of your unknown.

Data Table 4: Determining the Actual Concentration of the Unknown

Single Step Dilution for Final Answer			
Trial #	mL Unknown	mL H ₂ O	Absorption
1			
2			
3			

Table 17.4: Concentration Calculations for the Unknown

Post Lab Questions - Day 2

10. Using your Results from Data Table 4, and the equation of the line from your calibration graph, calculate the concentration of the diluted solution of your unknown. Show work!
11. Using the Results from Data Table 4, calculate the initial (undiluted) concentration of your Unknown using $M_1V_1 = M_2V_2$. Show work!
12. Suppose 300. mL of water was added to 50.0 mL your Unknown. What absorbance would you expect to find if the sample were placed in the Spectrometer? (Hint: you need to use both $M_1V_1 = M_2V_2$, and the equation from your calibration graph). Show work!

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Name: _____

Class: _____

Date: _____

Prelab Questions

1. Calculate the Molecular Weight of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$. Show Work.
2. Calculate the mass of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ required to make 100.0 mL a 0.150 M solution. Show Work.
3. Calculate the concentration of a solution made from 9.0 mL of 0.150 M $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ diluted to a final volume of 10.0 mL. Show Work.
4. Calculate the concentration of a solution made from 4.0 mL of 0.150 M $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ diluted to a final volume of 10.0 mL. Show Work.
5. Bonus: Using the data below, graph the absorption spectrum of the solution (Figure 17.1), and make a Beer's Law plot (Figure ??). At what wavelength does the Absorption Maximum occur? What is the value of the Absorptivity Constant?

Wavelength (λ) (nm)	Absorbance (A)
425	0.01
450	0.05
475	0.10
500	0.05
525	0.005
550	0.21
575	0.48
600	0.65
625	0.72
650	0.65
675	0.35
700	0.20

Concentration (M)	Absorbance (A)
0.035	0.42875
0.045	0.55125
0.055	0.67375
0.062	0.75950
0.075	0.91875

<subliminal message>Do the bonus, its easy and its fun!</subliminal message>

How many spelling errors did you find in the lab? Remember each spelling error or other correction is worth 1 pt of extra credit on the lab! (Offer valid only to the first person to report the correction).

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