Exp 01 - Spectroscopy

Getting started with solutions by learning how to use absorption spectroscopy to measure concentration.

Preparation *(complete this pre-lab work before coming to lab)*

Read the experiment Spectroscopy (http://chem220.com)

Start a new experiment in your lab book. Write the following:
- The experiment objective(s)
  (Objectives are the question(s) you are trying to answer with this experiment—be brief.)
- Your procedures as a short bullet lists of steps
- A simple table to capture the data you expect in your experiment.

Intended Learning Outcomes

* Provide and apply the definitions of solute, solvent and solution.

* Given a mass or number of moles of a solute and volume of solution, determine solution molarity.

* Use molarity as a conversion factor between moles of solute and volume of solution.

* Use the equivalence of volume $_{before}$ × molarity $_{before}$ = volume $_{after}$ × molarity $_{after}$ to solve dilution problems.

* Know Beer's law of absorbance, $A = \varepsilon \times l \times c$

* Convert between light absorbed and percent transmittance:

  * %Transmittance = $10^{(2 - Absorbance)}$

  * Absorbance = 2 - log (%T)

* From a Beer's law plot relating light absorbed to known concentration of different solutions determine the absorption coefficient ($\varepsilon \times l$).

* Using an absorption coefficient determine the concentration of an unknown solution from an observed absorption.

Report *(prepare a report on your experiment to be submitted in the following lab meeting)*

Prepare a report for this experiment according to this experiments report description for the parts we accomplished. Include the questions with answers for this experiment, except any your instructor tells you to omit.

Analysis *(calculations needed in your report analysis)*

Part A:

1. Calculate the absorbance values in part A.

2. Calculate the concentrations of solutions 2-6.

3. Prepare a plot of absorbance (y-axis) vs. relative concentration (x-axis).

4. Determine the best fit line through the data points.

5. Calculate the slope of this line, this is the absorption coefficient ($\varepsilon \times l$).

Part B:

6. Calculate the absorbance values of your unknown.

7. Use the absorption coefficient in part A, to determine the concentration of your unknown solution.

Post-Lab Questions *(rewrite these questions with your response and append them to your experiment report)*

(when calculations are used to produce your answer, show your calculations)

1. If 3.5 grams of carbon dioxide (CO_2) is dissolved in 1.5 L of water, what is the molarity of the resulting solution?

2. If 20.0 mL of 10.0 M ammonia (NH_3) solution is diluted to a new volume of 50.0 mL, what is the molarity of the new solution?

3. A student observes a given sample has a 32.5% percent transmittance (the intensity of the light shining through is 32.5% of what it is for pure solvent). What is the absorbance of this sample?
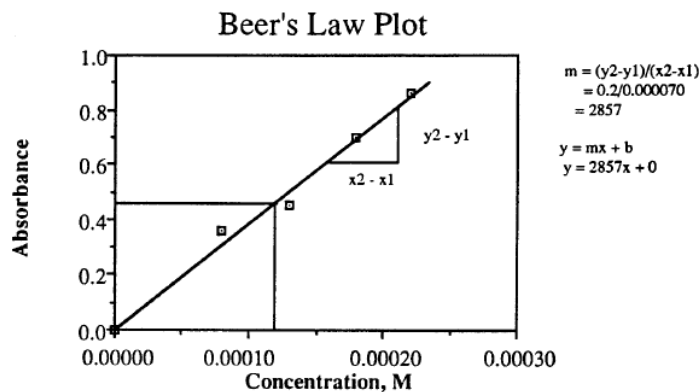
Beer's Law Plot

4. A student produces the Beer's law plot shown above, relating the concentration of a solute to the solutions absorbance. From the slope of the best linear fit to their data points the student determines the absorption coefficient ($\varepsilon \times l$) is 2857.

   a. If the student tested a sample that was 0.00015 M, what absorbance would they predict?

   b. What percent transmittance would the student expect to observe for this sample?

5. Given an unknown solution of the same substance used to produce the Beer's law plot above, the intensity of light shining through is only 45.7% of what it would be for sample of the pure solvent. What is the concentration of this solution?

INTRODUCTION TO SPECTROSCOPY

In **Part One** of this experiment you will be introduced to the fundamentals of spectroscopy. You will first learn how to properly use a Spectronic 20 instrument and then you will use the instrument to find the wavelength (λ) at which absorbance of light by a solution of a colored analyte has a maximum value (λ_{max}). In **Part Two** you will learn how a law of spectroscopy, termed Beer's Law, can be used to determine the concentration of a colored analyte in an *unknown* solution.
In this experiment you will do the following:

1. Learn to use the Spectronic 20, a spectrophotometer. (Part One)

2. Find λ_{max} for a colored analyte solution.(Part One)

3. Prepare colored analyte solutions of various known concentrations by diluting a stock standard solution. (Part Two)

4. Set the Spectronic 20 at the value of λ_{max} obtained in Part One, and then generate a calibration curve that relates absorbance to concentration. (Part Two

5. Use the calibration curve and the Spectronic 20 to determine the concentration of an unknown. (Part Two)

INTRODUCTION

The word **spectroscopy** is used to refer to the broad area of science dealing with the absorption, emission, or scattering of electromagnetic radiation by molecules, ions, atoms, or nuclei. Spectroscopic techniques are some of the most widely used analytical methods in the world today. *These techniques are useful in determining both the identity of unknown substances and their concentration in solution.* Different regions of the electromagnetic spectrum such as infrared, visible, ultraviolet, or, X-ray radiation can be used to interact with matter. The Spectronic 20 instrument you will use can correctly be called a **colorimeter,** because it measures the **absorption of** light in the **visible** spectrum that we perceive as color, and the technique used is said to be **colorimetric.**
The electrons and nuclei of atoms and molecules may exist in only certain specific energy levels, that is, they area quantized. They can absorb only photons having certain energies or wavelengths. The energies of light absorbed by a molecule can be related to motions (energy modes) of the molecule. A few examples are shown in Table I.

Table I.

Molecular Motion	**Absorbed Electromagnetic Radiation**	Energy
Rotation	Microwave, infrared	Low
Vibration	infrared	Moderate
Electron transitions	Visible, ultraviolet	High

Although the instruments that are used to measure the interaction of various regions of electromagnetic radiation with matter differ a great deal in design and operation, they all contain the same basic components. A schematic diagram of a simple instrument that is used to measure the absorption of visible light is shown in Figure 1.
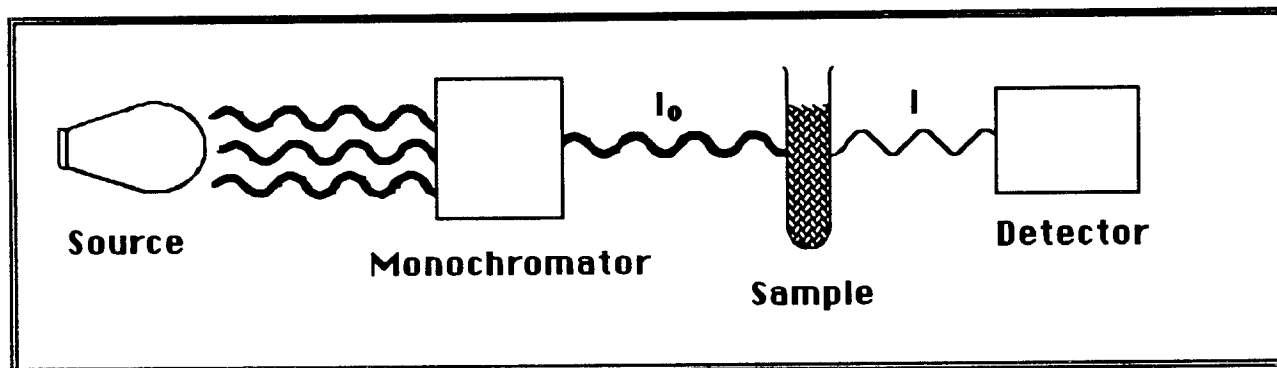

Figure 1.

The **source** provides the electromagnetic radiation that will be absorbed by the sample. It is often some sort of light bulb or lamp. The **monochromator** selects one particular energy (or wavelength or color) of light from the source. A prism, a diffraction grating or a colored filter together with slits and mirrors can serve as a monochromator. (A diffraction grating consists of a large number of closely spaced lines etched on a highly polished surface. The lines act as scattering centers for the incoming radiation, separating white light into the colors of the rainbow). The **detector** measures the amount of light that passes through the sample. A phototube (or photo cell) or photomultiplier is often used as a detector. All of these work on essentially the same principle. Light falling on the surface of the detector causes current to flow in a surrounding electrical circuit. The amount of current in the circuit is proportional to the amount of light striking the detector.

All the parts of the instrument work together as follows (Figure 1): Light from the source passes through the monochromator producing a beam with a single energy or a narrow band of energies. The intensity of this beam, I_0, is measured by the detector. The sample is then placed in the beam between the monochromator and the detector. If some of the light is absorbed by the sample, the intensity of the beam reaching the detector, I, will be less than I_0. The detector compares the two intensities and reports the result as either percent transmittance (**%T**) or absorbance (**A**).

These terms are defined to be:

$$\%T \ = \ I/\,I_0 \ x\ 100$$

(the fraction of I_0 that gets through the samples is called Transmittance)

$$A \ = \ \text{-}\log T \ = \ \text{-}\log(I/\,I_0) \ = \ 2 - \log(\%\ T)$$

If the monochromator is a prism or a diffraction grating, all of the energies (or wavelengths) are available and may be varied.

Molecules do not absorb all wavelengths equally well. Consider a colored object. Human sight is the brain's interpretation of photons of electromagnetic radiation in the Visual range (light) entering the eye. If all the energies (wavelengths, colors) are mixed they are perceived as *white* light. If no photons at all enter the eye we "see" black. A color is perceived if only photons of one energy (light of one color, monochromatic light) enters the eye or if photons of a complementary color are missing from the usual white light mix. A white object then appears to be white because it does not absorb any of the light that strikes it. A black object looks black because it absorbs all of the incident light. A rose looks red if it absorbs all the light except the red or if it absorbs the light of the color complementary to red - that is, blue-green. Table II shows a brief list of colors absorbed to give observed colors.

Table II. Visible Spectrum and Complementary Colors*

Wavelength , nm	Color (Absorbed)	Complementary (Observed)
400 — 435	Violet	Yellow-green
435 — 480	Blue	Yellow
480 — 490	Green-blue	Orange
490 — 500	Blue-green	Red
500 — 560	Green	Purple
560 — 580	Yellow-green	Violet
580 — 595	Yellow	Blue
595 — 610	Orange	Green-Blue
610 — 750	Red	Blue-Green

In order to achieve the most accurate results in Part Two in which we will determine the concentration of a colored species in solution, we need to maximize the absorptivity of the species (Part One). We must select the color (energy, or wavelength) of light from our source that is best absorbed by the molecule. For example, if a solution is green-blue, it will absorb orange light, and the wavelength of maximum absorbance, λ_{max}, will fall between 595 and 610 nm (see Table II above). Amounts of light absorbed, even in the case of concentrated solutions are very small compared to the amount of light available from the source. It is much easier for the instrument to "see" the change if we make it as large as possible. Most spectrometers have variable monochromators such as a prism or diffraction grating, making it is easy to select the complementary color from the source by stepping through the available wavelengths and plotting the absorbance (or percent transmittance) versus the wavelength manually or let the instrument scan through the range automatically.From the plot (spectra) we can determine the exact wavelength of the complementary color, which is the wavelength of the maximum absorbance (λ_{max}). Values for λ_{max} of many substances can be found in the chemical literature.

*Day, R. A., Jr., and A. L. Underwood. *Quantitative Analysis,* 3rd ed. (Englewood Cliffs, NJ: Prentice-Hall, Inc.), 1974.

PROCEDURE

<u>PART ONE</u>:
OPERATION OF THE SPECTRONIC 20 AND DETERMINATION OF λ_{max}

<u>Equipment Needed From The Storeroom</u>

2- Spec 20 tubes (cuvettes)

A. Operation of the Spectronic 20

The Spectronic 20 is a moderately expensive piece of scientific equipment and should be treated with all due care and respect.
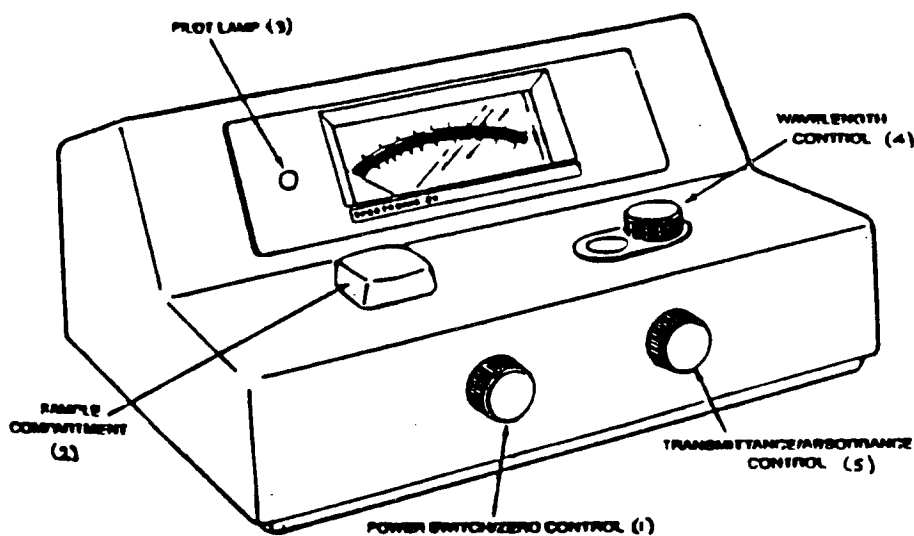

Figure II

In all the instructions that follow refer to Figure II.

Operating Instructions (Use with Parts One and Two Below.)

1. The Spec 20 is turned on by rotating the power switch, 1, until a click is heard and the pilot lamp,3, comes on. The instrument must war,m up for about 30 minutes prior to use.

2. Use knob 4, the wavelength control, to set the desired wavelength. (in Part One the first wavelength will be 400 nm; in Part Two, the wavelength you use will be λ_{max} that you determined in Part One.)

3. With the sample holder, 2, empty, close the door and set the readout scale to 0 % transmittance (absorbance) using knob 1. Be sure to line up the needle with its reflection in the mirror when reading the scale.

4. Obtain a Spec 20 sample tube. The tube may look like an ordinary test tube, but it is made of special high quality glass and is much more expensive. Clean the tube and rinse it with distilled water, and then fill the tube about 3/4 full **of** blank solution (the blank is distilled water in this experiment). **Carefully wipe away any solution and fingerprints from the outside of the tube using a Kimwipe.**

5. Open the lid of the sample holder, 2, and slide the sample tube into the opening. Find the hash mark (small straight line) on the tube and line it up exactly with a similar mark on the sample case. Close the door.

6. With the blank solution in the sample case, set the read-out scale to 100% transmittance (0 absorbance) with knob 5.

7. Remove the tube from the sample case and close the door. tf the scale does not read 0% transmittance, repeat step 3.

S. If it was necessary to adjust knob 1, repeat steps 6 and 7. Continue this process until consistent readings are obtained.

9. When consistent 0 and 100% readings have been obtained, set the sample tube containing the blank solution aside. **Be sure to save the blank until all measurements have been made.**

10. Clean another sample tube and rinse it with a small portion of the solution whose absorbance is to be measured. Then fill it with solution, wipe it with a Kimwipe and place it in the sample holder with hash marks aligned. **Read the percent transmiftance** and record the value. **Calculate the absorbance** from the percent transmittance using $A = 2 - \log (\%T)$.

11. Repeat step 1 0 for all solutions to be determined. When finished, clean and dry the sample tubes carefully and return them to the storeroom.

Note: The percent transmittance scale is linear. It is easy to read to – 0.01 units at any position on the scale (O to 100). The absorbance scale, however is logarithmic and reads from *right to* left. The graduations along the scale vary and cannot be read with as much accuracy as the transmittance scale. **Therefore, always read the transmittance scale and calculate the absorbance.**

Retain these instructions for use in future experiments with the Spectronic 20. It is not necessary to turn these pages in to your instructor for grading.

B. Deterrnination of λ_{max}

To determine λ_{max} you will begin by measureing the absorbance and transmittance at 400 nm, increase the wavelength by 20 nm, take another measurement, etc. until you have reached 680 nm. **A** plot of absorbance vs. wavelength will show you λ_{max}.

1 Designate one of the Spec 20 tubes as the blank by using a grease pencil to put the letter B on the upper part of the tube. Fill the tube with distilled water and set it aside.

2. Fill the second tube at least 3/4 full with one of the assigned colored analyte solutions to be tested. Set it aside.

3. Set the wavelength control at 400 nm, and go through instructions 1-10 for operating the Spec 20. When you have completed the instructions, you will have the first measurement for Table 1 on the next page.

4. Now set the wavelength control at 420 nm, and go through the instructions again. It is necessary to set the 0 transmittance with the sample compartment empty and the 100% transmittance with the blank *each time* you change the wavelength.

5. Continue to increase the wavelength by 10 nm and obtain transmittance and absorbance data until you have arrived at 600 nm.

6. Plot absorbance vs. wavelength (absorbance on the ordinate, wavelength on the abscissa) for the colored solution. Use an Excel spreadsheet for your plot. Your instructor will help you with this.

CLEANUP

1. Dispose of waste in the provided waste bottle.

2. Rinse and **dry** the Spec 20 tubes.

PART TWO: BEER'S LAW

Equipment Needed From The Storeroom

2 - clean cuvettes
1 - 10 mL graduated pipet
1 - pipet pump/bulb

Equipment Needed From Your Locker

1 - 50 mL beaker
6 - 18 x 150 mm test tubes
test tube rack

The amount of light absorbed is dependent on how well the substance absorbs light, the path length of the light, and the concentration. These parameters are combined into a mathematical relationship known as *Beer's Law.*

Beer's law is a relationship between the light absorbance of a substance and its concentration It is often written:

$$A = a.b.c$$

where

   A = Absorbance of solution
   a = absorptivity, or how well the substance absorbs light
   b = path length, length of sample holder (sometimes written as b)
   c = concentration of solution

Since **a** and **b** will not change in our experiment, we can combine them into a new constant, k. Thus: $A = kc$. Note that this is the equation of a straight line $y = mx + b$ where $y = A$, $x = c$, $m = k$ and $b = 0$. (Remember that m is the slope of the line, and **b** is the **y**-intercept.) Beer's law then states that a plot of absorbance vs. concentration will give a straight line passing through the origin. Such a graph is labeled a Beer s Law Plot . The slope of the line is characteristic of and depends upon the solution used.

Suppose that a series of solutions of some substance were prepared, each having a different known concentration. if the absorbance of each solution is measured at the same wavelength, and a plot is made of absorbance vs. concentration, a plot like the graph below should result. This figure is called a "Beer's Law Plot".

Beer's Law Plot

$m = (y2-y1)/(x2-x1)$
$= 0.2/0.000070$
$= 2857$

$y = mx + b$
$y = 2857x + 0$
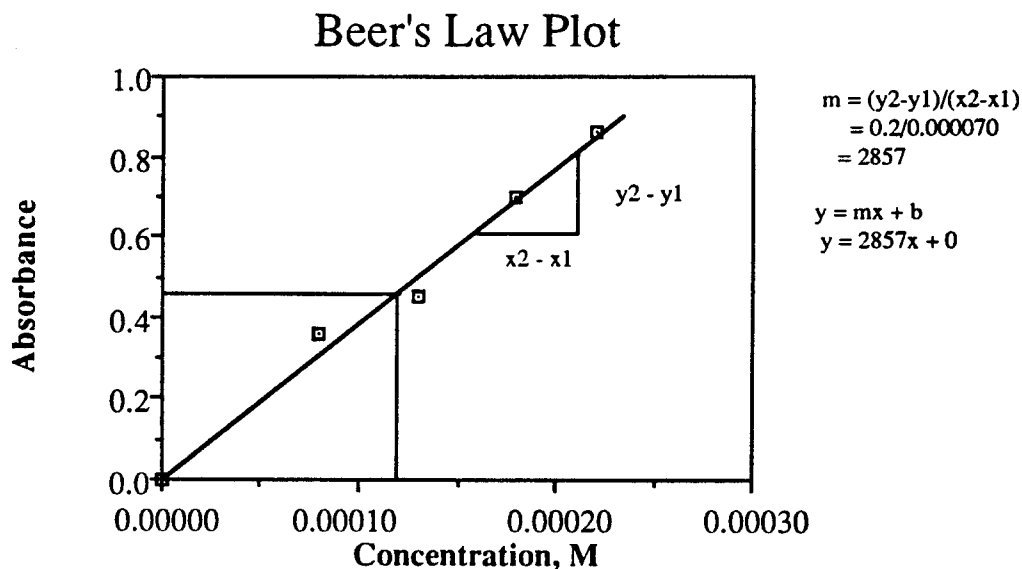
Note that few of the points, (if any) lie on the line. The line is a "best fit" - that is, the line is drawn closest to all of the points with the same number of points above and below the line. Although the line drawn is most accurate when the data points are processed statistically on a good hand-held calculator using the "method of least squares," one can get a very good approximation by holding a clear ruler on the data points and drawing a line which best averages the data.

By demonstrating a linear relationship between absorbance, A, and concentration, c, the Beer's Law plot not only confirms that the solution you are using conforms to Beer's Law, but it provides a calibration plot you can use to determine the concentration of a solution of the same substance of unknown concentration. For example, if you have an unknown which has an absorbance of 0.43, you would determine from your calibration curve that the concentration of the unknown was 0.00012 M (see graph above.)

A. Preparation of a Calibration Curve

1. Pour about 40 mL of your assigned stock colored analyte solution into a clean 50 mL beaker.

2. Label the 18 x 150 mm test tubes 1-6 using a grease pencil. Rinse the pipet with distilled water and pipet the volume of water as indicated in Table I into the test tubes.

3. Rinse the pipet with the stock solution, then pipet the volumes of stock solution as indicated in Table I into the test tubes.

Table I. .

Test tube #	mL of distilled water	mL of stock solution
1	10.0	0
2	8.0	2.0
3	6.0	4.0
4	4.0	6.0
5	2.0	8.0
6	0	10.0

4. Place a piece of parafilm over the top of tube 2 and shake to mix. Repeat for tubes 3-6.

5. Set the wavelength on the Spectronic 20 to the λ_{max} value which you determined in Part One. Place solution I (distilled water) into a Spec 20 tube to use as the blank to calibrate the Spectronic 20.

6. Empty the blank and condition it with a small amount of solution 2 (from test tube 2). Refill the tube with solution 2 and measure its transmittance. Record the value. Repeat for solutions 3 - 6 being careful to condition the cuvette before taking a transmittance reading.

7. Calculate the absorbance values using the mathematical equation given in Part One.

8. Calculate the relative percentage concentrations of solutions 2-6. Show your work for #2 as a sample calculation.

9. Prepare a plot of absorbance (y-axis) vs. relative concentration (x-axis) using an Excel spreadsheet. Use Excel to determine the best fit line thrugh our data points.

10. Calculate the slope, y intercept and correlatio coefficient of the best fit line using Excel. Record these values.

B. Determining the Concentration of an Unknown Solution

1. Obtain a sample of an colored analyte solution of unknown concentration (of the same color as that from which your calibration curve was prepared) and record its identification number.

2. Measure the absorbance of the unknown solution. Refer to the instructions in Part One of the experiment as needed. If another student has used the Spec 20 since you completed your measurements, re-calibrate the instrument before making any measurements. Again, use distilled water as the blank.

3. Use your Beers Law calibration plot to find the concentration of the unknown.

4. Clean and **dry** all glassware and return to the storeroom. (<u>DO NOT</u> use a test tube brush on Spec 20 tubes; it will scratch them.)

Name _____ Section _____

Table 1. Name & Color of Analyte Solution _____ λ_{max} _____

Wavelength , nm	% Transmittance	Absorbance

Name _____ Section _____

RESULTS

Name & Color of Analyte Solution _____

Test tube #	% Transmittance	Absorbance	Relative Concentration
1			
2			
3			
4			
5			
6			

Sample Calculation for the Relative Concentration of Test Tube #2

Calculation for slope of the calibration line

y-intercept _____

equation of the line _____

correlation coefficient

_____

Unknown Number	% Transmittance	Absorbance	Relative Concentration

Exp # _____

Chemistry Experiment Report

Name: _____

Student ID: _____

Class Section: _____

Experiment Title: _____

Unk#: _____

Bench / Locker : _____/_____

(write N/A if no unknown for this experiment)

This box is for use by your instructor.

_____ _____ _____
pre-lab   report   total

Unknown Composition or Identity:

A report must be submitted for all lab experiments conducted to receive credit for your participation. You may only report on experiments you conducted.

Reminder: All experiment reports should include each of the following sections. Each section should start on a new page and be clearly labeled.

➡ COVER PAGE (this page)
➡ 1. PRE-LAB
    ➡ Describe the objectives of this experiment (what you hope to conclude).
    ➡ Any pre-lab questions must be considered and answered before the start of the lab period. Your instructor will initial those pages and they must be included in your lab report to receive full credit for them.
    ➡ If there is a pre-lab quiz it will be graded and returned to you during that lab period. It should be attached to your report to ensure you receive full credit.
➡ 2. DATA
    ➡ Empirical data, only what you observed and others could if they repeated your experiment. Observations both qualitative and quantitative (measurements).
➡ 3. ANALYSIS (CALCULATIONS)
    ➡ Provide demonstrations (justification) of how your conclusions were developed from your observations. Most often accomplished with dimensional analysis or algebra (calculations).   For some experiments other methods will be demonstrated and need to be applied.
    ➡ It is only "known" if it can be justified … otherwise it's a guess.
➡ 4. CONCLUSIONS
    ➡ Your final answers. What you came to know by doing this experiment.   Be clear and brief, conclusions should not be more than one page and are often only a few sentences.
    ➡ If other factors effected your final answer, briefly describe them on this page and suggest how they may have effected your experiment.
➡ 5. QUESTIONS
    ➡ Attach and answer any post lab questions.

(download this page from your class website to print and attach to each report you submit)