

An Introduction to the Visible Spectrophotometer

INTRODUCTION

When light passes through a solution, not all of it is transmitted. If it is in a glass container, some light gets reflected by the container's inner and outer walls. Some may be scattered by suspended particles in the solution, making it appear cloudy. Some may be absorbed by molecules or ions in solution or by the solvent, making the solution appear colored. Color is the result of certain wavelengths of light being absorbed, while others are transmitted. The intensity of the color can be measured by comparing the amount of light passing through clear solvent to the amount of light passing through a colored solution at a specific wavelength in the visible region.

The outcome of this experiment is to use the intensity of color to determine the concentration of a solution. To accomplish this we will:

- Determine at what wavelength (color) the method is most responsive. This is where the color is most intense because the most light is removed.
- Learn the skills of dilution to create solutions of several concentrations.
- Establish a relationship between concentration and transmittance or absorbance.
- Use the relationship to analyze a solution.

A Visible Spectrophotometer is used to measure the amount of light transmitted (or conversely, absorbed) by solutions. A source (a light bulb) shines light down an optical pathway through a solution (in a "cuvette") and the light that passes through strikes a phototube. The electrical current out of the phototube is measured electronically and converted into a digital reading or a meter output. To make the technique quantitative, the output of the phototube with no light falling on it must be set to zero, and the output of the phototube from light of a specific wavelength passing through clear solvent (in a "cuvette") must be set to 100% "Transmittance". Therefore, the steps in the measurement are:

- 1) Have a stable source of light.
- 2) Select the wavelength of the light.
- 3) Calibrate the instrument:
 - a) "Teach" the instrument what no light is.
 - b) "Teach" the instrument how much light should get through clear solvent.
- 4) Measure the light passing through a solution at the same wavelength in the same (or an identical) cuvette.

Keep these steps in mind while you carry out the exercise.

Details of the instrument's operation are in its manual, and a quick guide is available with each instrument. Measurements are made either in "Transmittance" mode or "Absorbance" mode which are selectable outputs of the instrument. Alternatively, absorbance can be calculated from the equation

$$A = -\log (\%T / 100\%).$$

DILUTIONS

Dilution is a skill practiced throughout Chemistry. We will use it in this experiment to prepare solutions of several known concentrations. Concentrations can be expressed in any number of unit systems, such as grams per liter, or they can be unit-less, as in percent or in parts per million. Dilution techniques usually utilize either mass or volume measurements.

Look at the following example of a dilution and the calculation of the new, diluted concentration:

A solution was originally 1.00 gram / liter. 5.00 mL of the solution were taken in a pipet, and transferred to a graduated cylinder. Water was added to make the final volume equal to 50.0 mL.

Worthy of note:

- The small volume was measured using a highly accurate and precise piece of glassware.
- The volume of water added was not measured, but the final total volume was.

Calculation of concentration:

The original concentration is changed to a smaller value by a conversion factor called a “dilution factor.” This factor is the ratio of the initial and final volumes, i.e., (V_i / V_f). Note that the volume units cancel if the units are the same, in which case the concentration units do not change. The new concentration (C_f) is

$$C_f = C_i \left(\frac{V_i}{V_f} \right) \text{ where } C_i \text{ is the initial concentration. Therefore in our problem}$$

$$C_f = 1.00 \text{ g/L} \left(\frac{5.00 \text{ mL}}{50.0 \text{ mL}} \right) = 0.100 \text{ g/L}$$

This equation can also be used to calculate the volumes needed to carry out a specific change in concentration, and it is often expressed $C_i V_i = C_f V_f$. It is important to keep rigorous track of units to avoid unit conversion errors.

Doing the dilution:

- Choose glassware capable of the needed accuracy, but keep it practical.
- Measure the initial volume (e.g., with a pipet) and place it in the container used for the final volume measurement (e.g., a graduated cylinder or a volumetric flask).
- Add solvent (e.g. water) until the volume is brought up to the final mark.
- Transfer the solution to a labeled container for storage.

Name _____

Partner _____

Section number _____

Report: Introduction to the Visible Spectrophotometer**PROCEDURE:** (Pages 28 through 32 will also comprise your report.)

Record all data, with references to the page and letters of this procedure, in your lab notebook. Copy the format of the data tables into your notebook for use now, but **fill in the blanks on the report pages later.**

- A. Set up and calibration: Use the instrument according to the printed procedure. Obtain a set of two cuvettes. Use one of these **only** for deionized water. Calibrate the instrument at 425 nm using deionized water. (Part B may be done before the 30 minute warm-up period is complete.)
- B. Selection of optimum wavelength: Obtain about 30 mL of a solution of known concentration in a beaker. Label this “undiluted” and record its concentration.
1. Place a sample in a cuvette, read and record its %T at 425 nm.
 2. Remove the cuvette, and insert the cuvette that contains deionized water. Increase the wavelength to 475 nm and then reset 100% T.
 3. Exchange cuvettes and measure the colored solution’s %T at this wavelength.
 4. Continue this process in 50 nm steps until you read at 675 nm, **being sure to reset 100% T with deionized water each time you change wavelength.**
 5. Make a copy of the table below in your lab notebook. Examine your data and select two additional wavelengths 25 nm above and below the wavelength with the smallest %T. From the complete data set select the wavelength with minimum % T. This is the color where the most light is removed, where it has the maximum absorbance. You will use this wavelength for Part D.
 6. Compare this wavelength with one other group.

(Place data in lab notebook; fill these in later!)

Wavelength (nm)	% Transmittance
425	
475	
525	
575	
625	
675	

(For your report you will be asked to make a graph of these data.)

C. Preparation of diluted solutions:

Prepare three dilutions of the original solution. Each partner makes at least one dilution of the original solution. One partner should use a 5.00 mL volumetric pipet, the other a 10.00mL volumetric pipet for V_i . The third dilution should be made using 15.00 mL (10.00mL + 5.00 mL volumetric pipets) as V_i . Prepare 20 to 25 mL (V_f) of each. **Record the dilution procedure for your sample(s) and your partner's sample(s) in your notebook** and calculate the concentration of each. Record a sample calculation of concentration. Save each solution for Part D. You will have five samples for your data set: deionized water, three diluted samples of the original, and the undiluted original solution.

C_i (before dilution)	V_i (of undiluted solution)	V_f (of diluted solution)	C_f (of diluted solution)

D. Measurement of Transmittance and Absorbance vs. Concentration:

Set your instrument's wavelength to the wavelength you chose in Part B and record the wavelength in your lab notebook. Reset 100%T at this wavelength using deionized water. Then measure both % Transmittance and Absorbance at this wavelength for the least concentrated solution. (You do not need to remove the cuvette to "toggle" between % T and A.) Read the second dilution (working upwards in concentration) and then the undiluted solution. Finally, reset 100%T and read the undiluted solution again.

Wavelength _____ nm

C_f (record <u>all</u> values)	Sample	% T	A
0.000 g/L	Deionized water	100.0%	0.000
	Least concentrated dilution		
	More concentrated dilution		
	Most concentrated dilution		
	Undiluted solution		
	Undiluted solution		
	(Copy Part B data for this wavelength – see also* below)		

There were two measurements of the undiluted solution in this section. In the last row, copy the % T value for this wavelength from part B.

➤ *Show the calculation of A from $A = -\log(\%T / 100\%)$:

(This will give a total of three %T and three A values for the undiluted solution at this wavelength.)

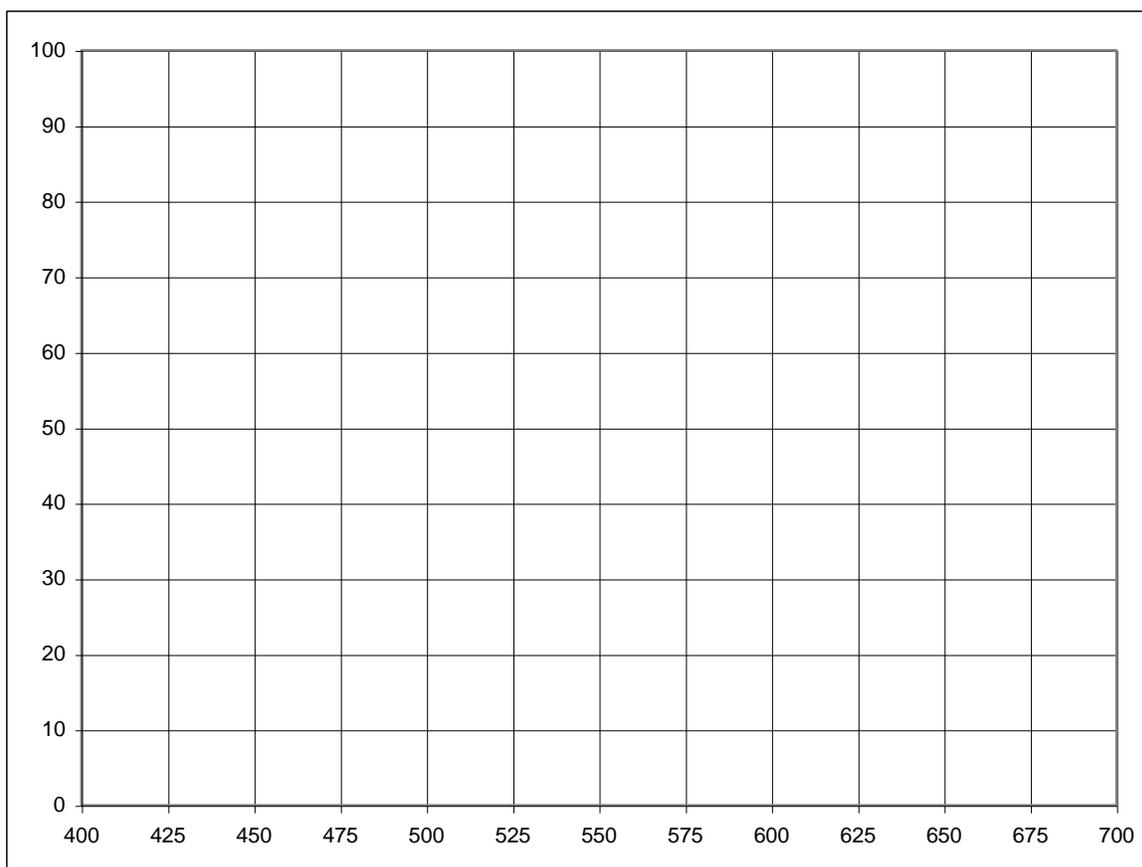
E. Unknown: Pick up one unknown solution, and read its %T and absorbance without dilution at the same wavelength as in part D:

Unknown's label number: _____ % T _____ A _____

F. Graphical Evaluations (These can be performed later.)

“Spectrum”

Plot all the data from the table in part B on the graph below, with wavelength on the x-axis and %T on the y-axis. Draw a smooth, curved line through the data showing how you think the overall spectrum would look, ideally. Label each axis and give the graph a title.



Concentration Study:

Plot all values of your % Transmittance and Absorbance data from the table from part D, each on one of the graphs on the next page. Use concentration along the X-axis and your results along the Y-axis. Be sure you have a linear x-axis and label the “tic” marks with concentration values. Include 100% Transmittance and 0.000 Absorbance at 0.000 concentration in addition to the other data points.

Label the graphs clearly, including the axes, and give each a title. Use a ruler to decide whether you think the relationship is a straight line (with a little “scatter”) or a curve. Connect the data points with either a smooth curve or with a single straight line fitted between the points. Do not use a series of point-to-point connecting lines, and do not obscure your data points with the line.

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POSTLABORATORY QUESTIONS

Complete and include all the data tables and graphs above. Answer the following:

1. When you made your graphs of Percent Transmittance vs. Concentration and Absorbance vs. Concentration, you included data points for 100% T at 0 concentration, and 0 Absorbance at 0 concentration. What in the experimental procedure made these valid data points?
2. Which of the two concentration graphs appears to represent a straight line function the best?
3. Write down a brief comparison of the two graphs: (a) their form, and (b) any conclusions they demonstrate concerning the effect of dilution:
4. An average deviation is a measure of how far (in absolute value) data are from their average: see the Appendix, "Making Use of Error Analysis", item C3. Using only the three undiluted solution measurements in part D, calculate the average absorbance and the average deviation of absorbance of the undiluted solution. Express the solution's average and average deviation in a confidence interval and also as a relative error (same Appendix, item B). *Show your calculation.*

Average absorbance = _____ \pm _____ relative error = _____

5. a) Use the best graph to determine the concentration of your unknown.

Unknown's Label: _____ Concentration: _____

Show this using a set of dashed lines on your graph.

- b) To estimate the \pm error in your unknown's concentration, use the graph from #5a) and determine how well you can estimate between the markings on the concentration axis. This is an estimate of the absolute error in the concentration. (Show how you made your estimate on the back of this page.) Express the concentration and error as a confidence interval:

Concentration = _____ \pm _____ relative error = _____

- c) Which is the "worse" error – the error in the spectrophotometer measurement (#4) or the error from how closely you could read the graph (#5b)? Justify your choice.

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Prelaboratory Assignment: Introduction to the Visible SpectrophotometerA. Use a textbook, a dictionary, or other sources for scientific definitions the following terms:

absorbance

cuvette

deviation

nanometer (nm)

phototube

solute

solvent

transmittance

B. Record the equation for dilution in your lab notebook under your preliminary entry for your lab date.

C. Assume that you have a solution in water that is 5.00 g/L. Describe the glassware, volumes, and the technique you would use to take a sample of this solution and make it into a solution of 0.200 g/L concentration. You are free to use any practical size volume measurements. Do you measure the amount of water added or the amount of final solution?