# Determination of Concentration by Spectroscopic Analysis — Lab 5

- **Students will work individually.**

## OBJECTIVES

- To create a set of standard solutions of known concentration by dilution from a stock solution.
- To measure the absorbance of the standard solutions with the LabQuest colorimeter.
- To determine the relationship between concentration and absorbance by creating a calibration curve.
- To use a measured absorbance to determine the concentration of an unknown solution using the relationship described by the calibration curve.

## INTRODUCTION

If you have ever made Kool Aid on a hot summer day, you have most likely been an unknowing investigator in a **spectrophotometry** experiment. Kool Aid powder contains sugar, flavoring, and coloring, among other ingredients. When mixing the powder with water, the strength of the drink can be estimated by its color. That is, the darker the color, the stronger the flavor. This is because as you add more Kool Aid powder to the water, you are increasing both the concentration of the flavored compounds and the concentration of the dye in the water. The higher the concentration of dye in the water, the less light passes through the drink and reaches your eye. Figure 1 below illustrates this principle, which provides the basis for the technique of spectrophotometry.

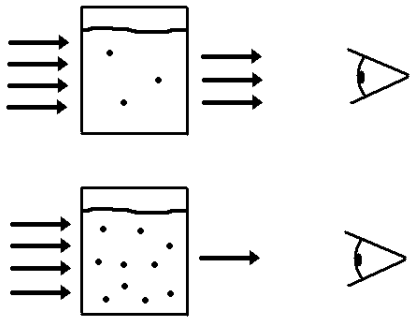

**Figure 1**: Transmitted Light versus Concentration

Spectrophotometry is an analytical technique by which an unknown concentration of a solution can be determined. This is possible due to light absorbing properties of the substance being studied, also known as the **analyte**. The spectrophotometer uses a beam of light covering a narrow range of wavelengths, i.e., of a well-defined color, which is directed through a sample tube in the sample holder and then onto a photoelectric detector, which measures the intensity of the beam (Figure 2). The measured intensity is displayed on a meter. In this lab, you will use a LabQuest handheld device with Vernier Software and Technology colorimeter. The colorimeter uses the same concept and similar technology as a spectrophotometer, but offers only fixed wavelengths.

To begin a measurement using a spectrophotometer or colorimeter, a volume of solvent is placed in a sample holder called a cuvette. This is known as a **blank**. The instrument is **zeroed** with the blank in the cuvette so that later, when measuring a solution containing analyte, any instrument response will

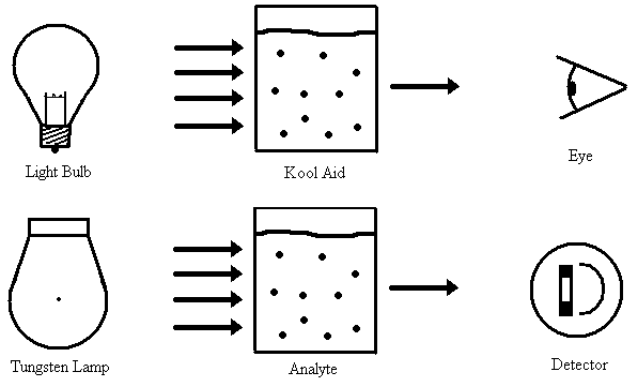


**Figure 2**: Detection of Light Passing Through a Solution

be due to the analyte as opposed to the solvent or cuvette. As the light passes through the sample some of the light is absorbed by the analyte, and the light leaving the sample cuvette is less intense than the light that passed through the blank. This difference is displayed by the instrument as an absorbance. Refer to Use & Theory of the Colorimeter in the Appendix for a more detailed discussion.

The characteristics of light absorption can be described by the Beer-Lambert law, which states that absorbance (A) by a chemical species in solution is directly proportional to three parameters: the concentration of the analyte (C), the path length of the light beam through solution (ℓ), and a characteristic of the absorbing species called the **molar absorptivity (**ε):

$$A = \varepsilon \cdot C \cdot \ell$$

Molar absorptivity varies strongly with wavelength, so a chemical species will absorb with differing strength at different wavelengths. Absorption is usually measured at a wavelength where the analyte has a strong absorption. This is done because a small amount of analyte still gives a relatively large instrument response, and the technique is more sensitive at that wavelength.

A compound or solution has a certain color for one of two reasons. First, the chemical could reflect light of one color. In this case, the species is the color of the reflected light. Alternatively, the species could absorb a certain wavelength of light, as discussed above, and transmit or reflect the remaining wavelengths. The solution then appears to be the **complementary color** of the wavelength that was absorbed. Complementary colors are found opposite each other on a color wheel (Figure 3). Thus a solution that absorbs blue light will appear orange; a solution that is green has absorbed red light.

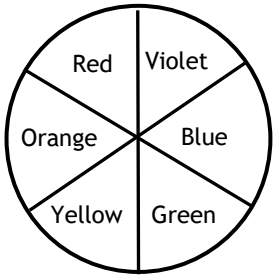


**Figure 3**: The Color Wheel

During the course of the experiment, the wavelength of light and the path length will be kept constant. Therefore, absorbance will vary with the concentration of analyte in solution. Again, an analogy to Kool Aid can be made: the greater the concentration of dye in the Kool Aid, the less light will pass through the drink and the darker the drink will appear to your eye. Similarly, the more concentrated the absorbing species, the less light will pass through the cuvette to the detector.

In this experiment, you will be making a calibration curve. The curve is a graph of the absorbances of a series of solutions of known concentrations. You can then measure the absorbance of a solution of an unknown concentration and calculate its concentration by comparing the absorbance value to your calibration plot. The analyte in this experiment is a common dye that is used in everyday products. The light absorbing properties of the dye responsible for its color are the same properties that will be used to determine the concentration of a solution containing this dye (hereafter referred to as the "unknown").

## PROCEDURE

You will use a stock solution, and prepare four dilutions of this stock solution, to create a calibration curve of absorbance versus concentration. You will then determine the concentration of an unknown by measuring its absorbance and comparing it to the calibration curve. You will share a Labquest Colorimeter with another student.

The following solutions will be provided for you:

- 3 dye stock solutions with labeled concentrations.
- 1 solution of unknown concentration per lab pair.

**NOTES:**

1. Most calculations required for this lab will be done during the lab period.
2. To use the LabQuest system, follow the procedure presented in this lab or use the procedure given in the Appendix. For an explanation of the function and operation of the LabQuest and the colorimeter refer to the Appendix.

### Part 1: The Unknown Solution

1. Obtain your assigned unknown solution from your TA and record any identifying information about it in the Data Section.
2. Compare the color of your unknown to the three example stock solutions that are provided in test tubes at the TA desk; note your observations in the Data section.

> ***Stop and Think #1****: How is comparing the intensity of color of your unknown to the intensity of color of the stock solutions related to what the spectrophotometer does?*
> (Answer this question in the Data section of your lab notebook.)

### Part 2: Creating the Standard Solutions

1. Based on a comparison of your unknown to the stock solutions, make a rough visual estimation of the concentration of your unknown. You will use this estimated concentration to decide on three dilutions of the stock solution to use, along with the stock solution itself, as your four calibration standards.

2. Copy the table below into the Data Section of your laboratory notebook and use it to calculate the information for your calibration standard solutions. Choose a stock solution that is clearly more concentrated than your unknown solution, and decide on the desired concentrations of the three calibration standard solutions that will be prepared by dilution of the chosen stock solution. Plan your standard solutions so that the concentration of your unknown is within the range of concentrations of your solutions. As an example, if the stock solution is 20 M and your unknown sample appears to be between 5 and 10 M, a good range of concentrations might be 20.0 M, 12.0 M, 8.0 M, 4.0 M.

| Calibration Standard | Concentration of stock solution to be diluted (M) | Required volume of stock solution (mL) | Molar concentration of calibration standard (M) | Final dilution volume (mL) | Required amount of dye (mol) | Absorbance |
|---|---|---|---|---|---|---|
| | **M1** | **V1** | **M2** | **V2** | | |
| **1 - stock solution** | | – | – | – | – | |
| **2** | | | | | | |
| **3** | | | | **25.00** | | |
| **4** | | | | | | |

3. From the desired concentrations of the calibration standard solutions, and the final total volume of 25.00 mL, calculate the required amount of dye (mol) and, therefore, the required volume of stock solution (mL) for each of the dilutions. Record these values in the table.

4. To make the calibration standards, pipet the calculated volume of the stock solution into a 25.00 mL volumetric flask and dilute to the line on the flask with distilled water. 10 mL serological pipets are available to use for measuring the stock solution. Repeat this process to make the remaining 2 dilutions.

**Part 3: Measuring the Absorbance of the Standard Solutions**

1. Press the power button on the LabQuest to turn it on. You will be sharing a colorimeter with another student.

2. Plug the colorimeter probe into one of the ports of the LabQuest hand held unit. A red box will appear on the screen that will display "CH#:Absorbance" and will show the currently read absorbance value.

3. Press the "<" and ">" buttons on the colorimeter to select the wavelength you will be using for your experiment. Set the wavelength to **635 nm.**

> ***Stop and Think #2****: Why are you using a specific wavelength of light to record absorbance measurements for the dye?*
> (Answer this question in the Data section of your lab notebook.)

4. Obtain four cuvettes from the TA, one for each of the five standard solutions. You or the other student sharing your colorimeter will also need one cuvette to use for the blank. Clean by shaking soapy water in them. ***Never use a brush to clean them as resultant scratches will interfere with light transmission***. Rinse well with tap water then distilled water. Invert to drain.

5. Fill the cuvette for the blank at least half full with distilled water and wipe off the outside with a Kimwipe. The purpose of a blank is to compensate for any light absorbed by the solvent so that absorption measurements made on the samples will measure the light absorbed by the solute only. Since water is the solvent for all the samples in this lab, water is an effective blank.

6. Place the blank in the sample holder of the colorimeter. The cuvette must be placed in the correct orientation for each reading; incorrect positioning leads to errors. Close the sample holder cover and press "CAL" on the colorimeter. Release the button when the red LED begins to flash. The absorbance should be 0.000 or 0.001. When the LED stops flashing the calibration is complete. Retain this cuvette as the colorimeter "zero". It should be re-checked periodically

throughout the experiment. It must be checked with each series of measurements as well as whenever the wavelength is changed.

7. Measure the absorbance of each standard solution: Rinse a cuvette with a small amount of the solution to be measured and discard it into a waste beaker. Fill the cuvette with the solution to about ¾ full, wipe the outside of the cuvette with a Kimwipe. Place the cuvette in the sample holder of the colorimeter, making sure the cuvette is properly aligned, and close the cover.

8. When the absorbance reading on the LabQuest display has stabilized, record the absorbance value in the Standard Solutions Data Table. Save the cuvette with this standard solution in case repeat measurements are needed.

9. Measure the absorbance for the remaining three standard solutions. Close the colorimeter sample holder cover while you prepare each of the remaining cuvettes.

**Part 4: The Draft Calibration Curve**

At this point it is important to check the quality of your data before you use it to determine the concentration of your unknown solution. Insure that your measurements are yielding a linear calibration curve by preparing a draft calibration curve as described in this section.

Sketch a calibration curve in the Calculations section by plotting Absorbance vs. Standard Solution Concentration. Ensure that your graph is reasonably linear; if not identify the problem and repeat a measurement or remake a standard solution.

**Part 5: Measuring the Absorbance of the Unknown Solution; Estimating the Concentration**

1. Check for zero absorbance when the blank is in the sample holder.

2. Discard the standard solution in the middle of the concentration range in order to have a cuvette for the unknown. Rinse with DI water and invert to drain. Use a small portion of your unknown solution to rinse the cuvette; discard this portion. Fill the cuvette about ¾ full with the unknown solution; measure and record the absorbance.

3. Using your draft calibration curve, estimate the concentration of the unknown based on its absorbance value. If the concentration does not fall within the range of your standard solution concentrations, consult with your TA as to the possible error.

4. When you have completed all measurements and your results are reasonable, have your TA check your data and calculations through Part 5.

5. Clean up: Clean and rinse the cuvettes (**remember, no brushes**) and return them per the TA instructions. Turn off the LabQuest and remove the colorimeter.

**Part 6: Determining the Concentration of the Unknown**

1. Prepare a software-generated graph of Absorbance vs. Standard Solution Concentration. Include the equation of the line on the graph.

**2.** Calculate the concentration of the unknown.

**STUDENT ASSIGNMENTS**

**Pre-laboratory Preparation – Complete Before Coming to Lab**

**A. Pre-Lab Assignment** – Complete the pre-lab assignment in LabFlow.

The following study questions are not graded but will help you prepare for the pre-lab assignment.

**Part 1 : PhET Simulation Pre-lab Activity**

The Beer's Law Lab PhET simulation models the experiment you will be doing in this lab. Use the simulation to answer the following questions for your pre-lab, and to familiarize yourself with concepts you will use throughout your lab work and lab report.

A. Download the sim: http://phet.colorado.edu/en/simulation/beers-law-lab

B. Explore all of the controls in both tabs of the sim for 5 minutes.

**Concentration Tab**

**PhET 1.** Record 2 (or more) ways that you can accomplish the following actions in the simulation:

- Change the solution volume
- Change the number of moles of solute
- Change the molarity of the solution

**PhET 2.** How are moles, volume, and molarity related?

**Beer's Law Tab**

Consider Beer's Law: $A = \varepsilon C \ell$

(A = absorbance, ε = molar absorptivity, C = concentration, and ℓ = path length)

**PhET 3.** Explain the relationship between A and C, using evidence from the simulation.

**PhET 4.** Based on Beer's Law, would you expect the wavelength used to affect your absorbance versus concentration relationship? What do you observe in the simulation?

**Part 2 : Pre-lab Questions**

1. Describe how to make the following solutions using a solid (MM = 646.38 g/mol) and distilled water: (Hint: molarity (M) = moles solute/L solution)

   a. 25 mL of 0.0004 M

   b. 50 mL of 0.0001 M

   c. Describe how to make the same solutions as in 2a and 2b but by diluting a 0.0005 M stock solution. (Hint: $M_{stock} \times V_{stock} = M_{dilute} \times V_{dilute}$)

2. Using the data below, generate a calibration curve using graphing software. Plot molar concentration on the x-axis and absorbance on the y-axis. Fit a linear trendline to the data points and include an equation and $R^2$ value on the chart.

| Calibration Standard | Molar Concentration (M) | Absorbance |
|---|---|---|
| 1 | 0.00020 | 0.40 |
| 2 | 0.00012 | 0.25 |
| 3 | 0.00008 | 0.16 |
| 4 | 0.00004 | 0.08 |
| 5 | 0.000016 | 0.04 |

**C. Prepare the Data Section -** Prepare the Data Section of the notebook. Format this section for the collection of data and observations during the lab, as shown on the next page of this Lab Manual. The Data Section must be formatted before coming to lab.

**II. Data and III. Calculations -** In your lab notebook, format the Data and Calculations Section as shown below. Show an example of each calculation.

**Part 1: The Unknown Solution**

Unknown identifier label (a number or letter):

Observations of the color of your unknown in comparison to the stock solutions:

Answer the *Stop and Think* question #1:

**Part 2: Preparing the Standard Solutions** – Copy the following table into your lab notebook. Enter data as described in the Procedure Section; the last column will be completed in Part 3.

| Calibration Standard | Concentration of stock solution to be diluted (M) | Required volume of stock solution (mL) | Molar concentration of calibration standard (M) | Final dilution volume (mL) | Required amount of dye (mol) | Absorbance |
|---|---|---|---|---|---|---|
| | **M1** | **V1** | **M2** | **V2** | | |
| **1 - stock solution** | | **–** | **–** | **–** | **–** | |
| **2** | | | | **25.00** | | |
| **3** | | | | | | |
| **4** | | | | | | |

**Part 3: Measuring Absorbance of the Standard Solutions** – Measure the absorbances of the Calibration Standard Solutions as described in the Procedure Section, and enter the values in the table.

Answer the *Stop and Think* question #2:

**Part 4: The Draft Calibration Curve** – Create the draft Calibration Curve as described in the Procedure Section; allow adequate space in your lab notebook.

**Part 5: Measuring the Absorbance of the Unknown Solution; Estimating the Concentration**

Absorbance of the unknown:

Estimated concentration from the calibration curve:

Note: DATA AND CALCULATIONS FOR PARTS 1–5 MUST BE CHECKED BY YOUR TA BEFORE LEAVING LAB.

**Part 6: Determining the Concentration of the Unknown -** In Excel, create a plot of Absorbance of each Calibration Standard on the y-axis versus Concentration of the Calibration Standard on the x-axis. Include a title, axes labels with units, a trendline and the equation and $R^2$ value for the trendline. Print the plot and include it with your report.

Calculate the concentration of the unknown solution using the absorbance value and the equation for the trendline fit to your calibration curve. Show the calculation.

**IV. Post-Laboratory Assignment**

Complete the Post-Laboratory assignment in LabFlow.