Matter and Motion
Fall 2015
Chemistry Lab 3: Concentration and Absorption Spectroscopy
Adapted from Dr. Dharshi Bopegedera, TESC

Overview

The goal of this lab is to determine the molar concentration of an unknown dye solution using absorption spectroscopy. To achieve this, we will use volumetric glassware to accurately prepare stock solutions of known concentrations and use the stock solutions to make a Beer's Law plot. A Beer's Law plot gives the linear relationship between absorbance (A) and concentration as described below.

Beer-Lambert's law relates the absorbance of light passing through a medium (a solution, in this case) to the path length and concentration as follows.

$$A = -\log\left[\frac{I}{I_0}\right] = \varepsilon_\lambda C L \qquad \text{Equation 1}$$

A = absorbance
ε_λ = molar absorptivity coefficient (or molar extinction coefficient) which is wavelength dependent
C = concentration of the sample (a solution, in this case)
L = path length through which light travels
I = intensity of the transmitted radiation
I_0 = intensity of the incident radiation

Transmittance (T) is defined as $T = I/I_0$

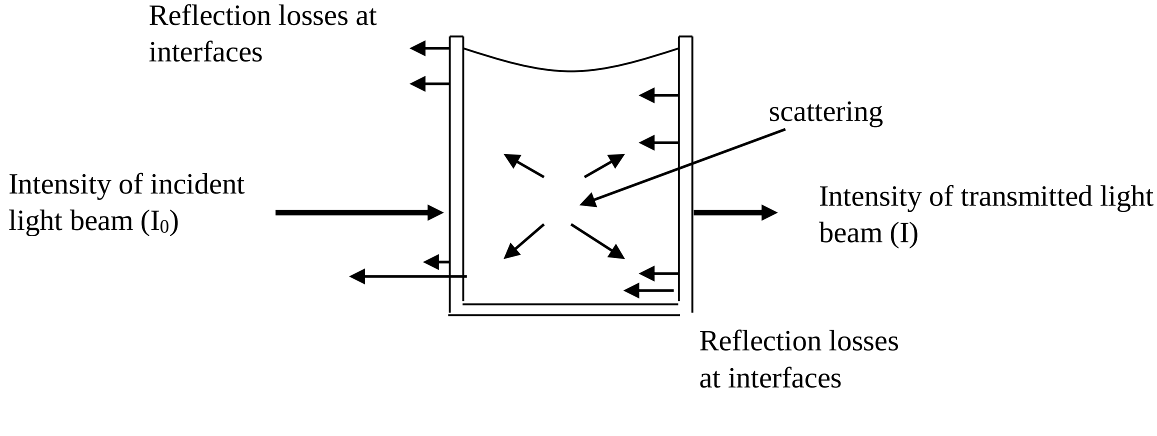
Notice from the Beer-Lambert's law ($A = \varepsilon_\lambda C L$) that absorbance is:

- directly proportional to concentration (C)
- directly proportional to path length (L)

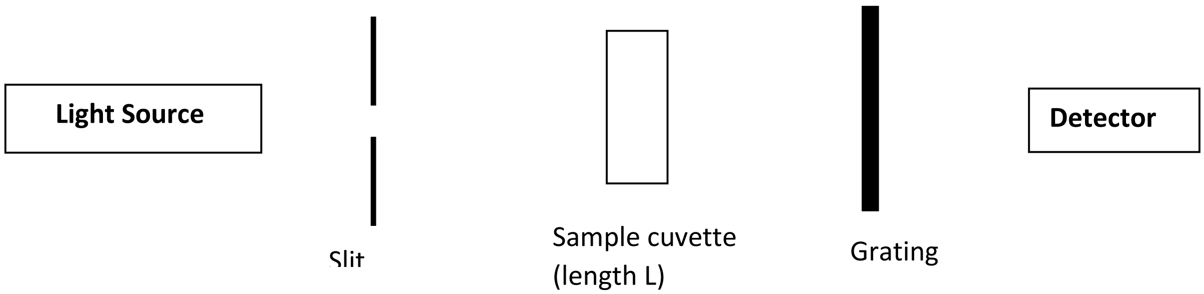
Therefore we can increase the absorbance by either increasing the concentration of the sample or by increasing the path length (since molar absorptivity coefficient is an inherent property of matter and we have no way of changing it). At high concentrations of the sample however, deviations from the direct proportionality between absorbance and concentration occur frequently. Therefore when recording absorption spectra we use samples of low concentration so that absorbance stays below 1.

Absorption Spectroscopy

Light can be absorbed, transmitted, reflected and scattered when passing through a solution. These processes are shown in the following diagram.



Block Diagram of a Spectrophotometer



In this lab we will observe absorption spectra, which are graphs of absorbance versus wavelength. The spectrum tells us what wavelengths of light are absorbed by a given sample.

Note that

$$A = -\log\left[\frac{I}{I_0}\right]$$

The spectrophotometer records I and I_0. Then it computes the value of absorbance (A) using the above mathematical relationship.

PRE-LAB QUESTIONS

Read the lab carefully (including the Post-lab) then complete the Pre-lab before coming to lab. It should be completed in your chemistry lab notebook.

1. Some of the words in this lab may be new to you. Make a list of any new vocabulary and their definitions.
2. Describe all of the data/measurements you will take during this lab. Be as specific as possible, i.e, include the units used and the number of times each measurement made.
3. Explain the process of making a Beer's Law plot and how it will help us determine the concentration of an unknown solution.
4. The red dye we will use in this lab is called Allura Red AC. It has a chemical formula of $C_{18}H_{14}N_2O_8S_2Na_2$. A) Calculate the molar mass of Allura Red AC. B) Calculate the number of grams of Allura Red AC in a 50 mL solution with a concentration of 0.0500 mole/liter. Please show your work.

EXPERIMENT PROCEDURE

No partners for this lab. You must make your own stock solutions and your own absorbance measurements.

PART I – Standard Solutions and Beer's Law Plot

1. Record the size of your volumetric glassware (burette and volumetric flask) and note the precision if it is specified. Record volumes in this lab to the 100^{th} of a mL.
2. Collect the stock solution in a beaker and rinse your burette with a small portion. Make sure to record the concentration of the stock solution. Note any additional observations.
3. Carefully fill your burette and then drain a little liquid to ensure there are no bubbles in the valve or tip area. Make sure your initial volume is "below" the 0.00 mL mark.
4. Prepare standard solutions of the desired concentrations in the volumetric flasks. You will have 5 standard solutions. See the table below for approximate volumes of DI water and stock solution to use in each of your standard solutions. You do not need to use these exact volumes, but you do need to record

the exact volume that you use. Note that the data for each volume dispensed from your burette **must have an initial and final volume reading**. You may want to prepare a table with columns for solution #, initial volume, final volume, and dispensed volume to help you stay organized. The actual volume dispensed is calculated from the difference between the initial and final readings. After preparing 50.00 mL of the first standard solution in the volumetric flask, put the solution in its own (clean!) labeled beaker. Make sure to rinse your volumetric flask thoroughly with DI water between preparing each solution. It does not need to be dried. Why?

Solution Number	stock red dye solution (mL)	DI H_2O (mL)
1	3	47
2	6	44
3	12	38
4	25	25
5	50	0

5. Turn on your spectrophotometer and select “wavelength scan.”
6. Before measuring the absorbance of your dye solutions, you need to record a “blank spectrum” of DI water in a cuvette. Make sure the outside of the cuvette is clean and dry before your measurement.
7. Use a Pasteur pipette to transfer your 1st stock solution to the cuvette and record the absorbance (A) at the peak wavelength (probably close to 530 nm) for each of your standard solutions. The reading for wavelength and A can be found in the lower-right corner of the screen. Record both the wavelength and the absorbance. Make sure to thoroughly rinse and dry your cuvette between solutions. Make sure to start with the lowest concentration solution to minimize the chance of altering your known concentrations. Prepare a table with columns for solution #, absorbance, and concentration. Repeat the absorbance measurements for the remaining 4 standard solutions.
8. Calculate the concentration (in moles/liter) of your five standard solutions. Please show your work for at least the first calculation.
9. Open Excel and create a Beer’s Law Plot by plotting your concentration and absorbance data. Discuss at your table which variable is the independent variable.

PART II – Known and Unknown solutions

10. Collect a “known” solution sample and record the absorbance at the peak wavelength. Make sure to record the concentration of the known.
11. Collect an “unknown” sample and record the absorbance at the peak wavelength.

CLEAN UP

- The dye solutions can be disposed of down the drain.
- Rinse your glassware and place it in the appropriate location for drying.
- Clean and dry any spills in your area and/or around the sinks.
- Ask Sina for a community job.

Continue to the Post-lab if time allows.

POST-LAB: Calculations and Analysis
Please show your work and keep track of units.

1. At what wavelength was the maximum absorbance of the dye solution? What color does this wavelength correspond to? What was the color of the dye solution to your eye? Comment on the relationship between the color a substance appears and the color it absorbs.

2. Explain the importance of recording a blank spectrum before measuring the absorbance of the dye solutions.

3. Use your concentration results (Lab Part I, step 8) to calculate the number of moles of dye present in 50 mL of *one* your diluted standard solutions.

4. Use the molar mass of Allura Red AC you calculated in the Prelab along with your answer from Postlab question 3 to calculate the grams of dye present in 50 mL of your diluted standard solution.

5. Use the equation of the line from your Beer's Law plot to calculate the concentration of the provided *known* and *unknown* solutions.

6. Summarize your results for the following:

- Actual concentration of the known
- Calculated concentration of the known
- Calculated concentration of the unknown

7. Calculate % error between the calculated concentration of the known and the actual known.

8. Discuss the reliability of your assessment of the unknown concentration. Use evidence from your results to support your claim. Comment on any possible systematic errors in this lab and potential ways to improve it.