Absorption Spectrophotometry Introductory Laboratory

The first laboratory is designed to accomplish three things: (1) practice with quantitative glassware such as volumetric pipets and flasks; (2) an introduction to a more problem-based learning approach in the laboratory; and (3) an opportunity to write and receive feedback on a thorough laboratory report. This last point is important since this is a writing intensive course. Details concerning the lab write ups can be found in the ACS Style Guide and on the instrumental website. In addition you can use this lab to submit to your Electronic Writing Portfolio (EWP) since this is the only lab you will submit a draft for feedback. Details on this requirement can be found at the Center for Academic Support & Achievement in 9th Street Hall Room 3001, and at www.eiu.edu/~assess.

Overview

You will be given a solution of a specific water soluble dye of unknown concentration. You will know what the dye is, and solid dye will be available for your use. Your assignment is to determine the concentration of the dye solution using visible absorption spectrophotometry.

General Procedure

Discuss with your group and instructor if necessary the approach you will need to take to determine the concentration of the dye solution. What do you need to know to lay out a possible procedure? How could you obtain the required information? Might it be most efficient to do some preliminary work prior to setting up an elaborate procedure? You may find it useful to first review a general discussion of absorption in Harris, Chapter 18. Remember that in spectrophotometry Beer’s Law rules: \( A = ebc \), where \( A \) is the absorbance, \( e \) the molar absorptivity (wavelength dependent), \( b \) the path length, and \( c \) the concentration of the solution.

Application

The spectrophotometer is an important analytical instrument that makes possible a quantitative measurement of light passing through a clear solution. The first step in such an analysis is to determine the optimum wavelength (i.e. color of light in visible spectrophotometry) to use in the analysis. The wavelength of light chosen must be appreciably absorbed by the analyte under analysis, for otherwise a measurement of transmitted light would not be a significant measure of analyte concentration. On the other hand, the analyte must not absorb too much of the wavelength chosen, for there might not be enough transmitted light to measure accurately. The best compromise between too much and too little absorption comes in the region between 0.1 and 1 absorbance units.

This, however, is not the only consideration. The wavelength of light that is chosen must fall in a region where the %T is not rapidly changing with wavelength. This is because the spectrophotometer cannot
isolate a single wavelength but isolates a band of wavelengths. If all the wavelengths in this band are absorbed to nearly the same extent by the analyte, then the situation is nearly the same as when one is able to isolate a single wavelength. Thus, flat portions of the Absorbance vs. wavelength plot (the absorption spectrum) are selected for analysis, usually the top (wavelength of maximum absorbance) of the absorption peak. This has the added advantage of providing the greatest analytical sensitivity.

**Additional information**

Dyes are very light absorbing so start with a very dilute solution, less than millimolar. Remember you don’t have to hit a desired concentration exactly, but you should know the exact concentration. This concept will save you time. The molar masses are as follows:

- Eosin Yellow = 691.86 g/mol
- Bromphenol Blue = 669.97 g/mol
- Malachite Green = 364.92 g/mol
- Methyl Orange = 327.34 g/mol

The unknown concentration must be within the range of the concentration of your standards. Note from the introduction that there is an optimum absorbance “window” for a Beer’s Law type of analysis. This corresponds to an optimum concentration “window” for standards and unknown.

From your data you will make a plot of absorbance versus dye concentration. Use ppm (parts-per-million) for concentration units. In aqueous solution, a ppm is 1 mg of analyte/Liter of solution (why?). Use linear least squares to analyze your calibration data. Also, propagate the uncertainty of your analysis from the linear least squares data. This was all done in quantitative analysis, if you have trouble let us know. Remember, an analytical result is meaningless without an adequate uncertainty estimate.