Absorption Spectrophotometry and Calibrations II – Standard Additions

Overview

Analogous to the first week’s lab, 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. This week there is an additional complication: the solvent is not pure distilled water. The solution will contain other solute(s) of unknown composition which interfere with the analysis; these other solute(s) also absorb at the analysis wavelength. This is an example of a matrix effect.

General Procedure

This is a spectrophotometric analysis, so Beer’s Law remains applicable. However the use of a simple calibration line to determine the unknown concentration is not applicable. A blank cannot be prepared since the matrix [interfering substance(s)] is unknown: the matrix absorbs at the analytical wavelength. A normal calibration line will result in an underestimate (negative bias) of the unknown concentration, since the unknowns prepared in pure water cannot mimic the extra absorbance from the matrix that is not attributable to the analyte. This common problem is readily addressed using the method of standard additions. By “spiking” the sample with additional analyte under carefully controlled conditions, a calibration line can be obtained by plotting the concentration of added analyte on the x-axis vs. absorbance on the y-axis. The original analyte concentration is then determined by extrapolation of this data (mathematically, not graphically) to determine the analyte concentration in the original unknown. Note that on page 89 Harris (7th Edition) states that the added standard should increase the signal (i.e. the absorbance in this case) by a factor of 1.5 to 3.

There are some guidelines which should be adhered to, as in the first week’s lab, as they are applicable to all absorbance spectrophotometric measurements. The unknown concentration must be within the range of the concentration of your standards. Absorbance measurements must lie between 0.1 and 1 absorbance units for maximum precision and accuracy. The measurements are best done at the top of the analyte’s absorbance peak for maximum sensitivity and minimum error.

The two texts explain standard additions differently, but the approaches are equivalent. The Harris text describes the standard additions technique in Section 5-3 pp. 87-90, and the Skoog text describes this technique in Section 1D-3 pp. 13-17. An informed approach to this lab may be obtained by studying these sections of the textbooks beforehand. Ask your instructor if there are questions, (s)he will be impressed that you have studied the material!

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

From your data you will make a plot of absorbance versus dye concentration. Use ppm (parts-per-million) for concentration units. Use linear least squares to analyze your calibration data. Also, propagate the uncertainty of your analysis from the linear least squares data. Although the “extrapolation method” to propagating uncertainties is superior here, stick with the “algebraic method” (J. Chem. Educ. 1999, 76, 805). Since $c_x = b/m$ (where $y = mx + b$), the relative variance in $c_x$ is equal to the sum of the relative variances of $b$ and $m$, both of which can be obtained from linear regression data.