

CHEM 334 Quantitative Analysis Laboratory

The Methods of Calibration Curve and Standard Addition

Introduction

One of the principle activities in the Quantitative Analysis Laboratory is the measurement of the concentration or total quantity of an analyte in an unknown sample. This activity can be accomplished by the measurement of some property of the analyte, for example, absorbance or emission, using a spectrophotometer or some other comparable instrument. It is generally necessary to calibrate the instrument or perhaps the entire measurement process including the instrument. This is most often performed by comparing the measurement of the unknown sample to the measurement of each of a set of prepared samples with known concentrations of the analyte. The results of the measurements can be plotted against the known concentrations to create a **calibration curve**. This relationship is often subjected to a mathematical analysis where the experimental calibration data is fitted to a linear expression that is easily inverted whereby the unknown concentration can be determined by evaluation of the inverted expression together with the sample's measurement value. There are circumstances when the use of a calibration curve is additionally required because the relationship between concentration of the analyte and the instrument response is non-linear. In these cases the mathematical expression is more complex and various specialized methods are used to invert or approximate it.

Under favorable circumstances only a single measurement of the unknown sample is required as described above. In other cases where there is an interaction between the analyte and one or more of the other components of the sample (collectively known as the **matrix**) that distorts the unknown sample measurement and renders the calibration curve irrelevant. In these cases it is necessary to make a set of samples containing identical quantities of the unknown sample and systematically varied known concentrations of the analyte. The resulting several measurements are then analyzed either graphically or preferably analytically to yield a measurement of the unknown sample. This method is known as **standard addition**.

In this experiment disodium erythrosin ($C_{19}H_6O_5I_4Na_2$, FW 879.9 $g\ mol^{-1}$) will be used together with a visible spectrophotometer for a measurement of optical absorption. The molecular formula of disodium erythrosin is shown in Figure 1.

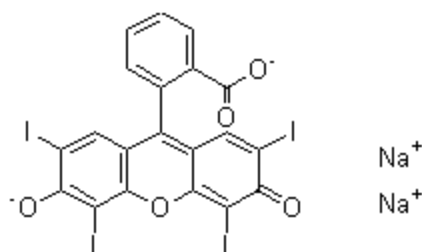


Figure 1. The molecular formula of disodium erythrosin.

Disodium erythrosin (hereafter erythrosin) exhibits a visible spectrum with a (strong) maximum absorbance (λ_{\max}) at a wavelength of 526 nm. The absorbance at this wavelength will be used to estimate the concentration of erythrosin in aqueous solution.

The basis for spectroscopic analysis is the relationship used in this measurement is known as Beer's Law or the Beer-Lambert Law (**Equation 1**).

$$abs = Abc \quad (1)$$

Where abs is measured absorbance, A is the molar absorptivity of the analyte, b , is the length of the path that is fixed at one centimeter and c is concentration of the analyte. This equation states that there is a linear relationship between the absorbance of light and the concentration of the absorbing species. Instrumental limitations and multiple absorption phenomena can become important at high values of absorbance and cause the relationship to become non-linear. The upper limit of the linearity calibration must always be established by experiment.

A representative calibration curve of a hypothetical substance displaying absorption at 562 nm is shown in Figure 2. Included on this figure is "curve fit" (via the method of linear regression) of the experimental data to a linear expression together with the derived values of slope and intercept with their uncertainties expressed as one standard deviation.

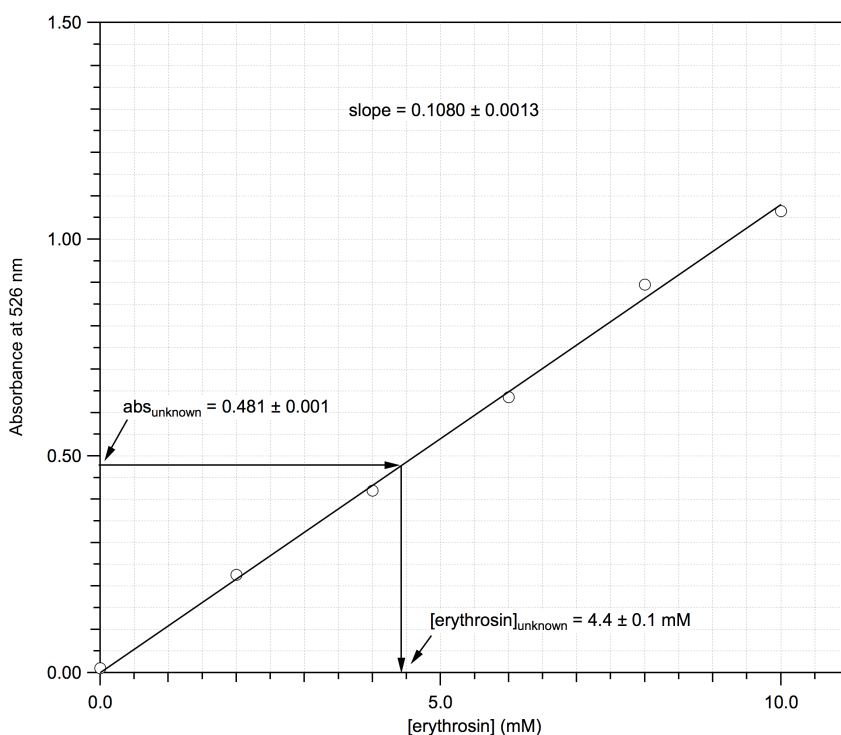


Figure 2. The calibration curve of a hypothetical substance with a molar absorptivity of $1.080 \pm 0.0013 \times 10^{-4} \text{ M}^{-1} \text{ cm}^{-1}$. The slope and y-intercept of the linear expression fitted to the experimental data are shown with their derived uncertainty expressed as one standard deviation. The absorbance of 0.481 ± 0.002 of a solution of this substance is shown also together with the graphical estimation of the substance's concentration of $4.4 \pm 0.1 \text{ mM}$.

The graphical estimation of the concentration of the same substance by measurement of the absorption of a solution of an unknown is also shown on Figure 2.

The curve fit of the experimental data yields the following expression:

$$abs = (1.080 \pm 0.0013)[erythrosin] \quad (2)$$

This expression can be easily inverted to give:

$$[erythrosin] = abs / (1.080 \pm 0.0013) \quad (3)$$

Standard techniques for the propagation of uncertainty can be applied to complete the uncertainty analysis.

A representative standard addition curve of the same hypothetical substance is shown in Figure 3 with its fitted linear expression and with the experimental data and corresponding fitted linear expression from Figure 2. Also included in this figure is a graphical estimation of the concentration of the substance using the value of the x-intercept.

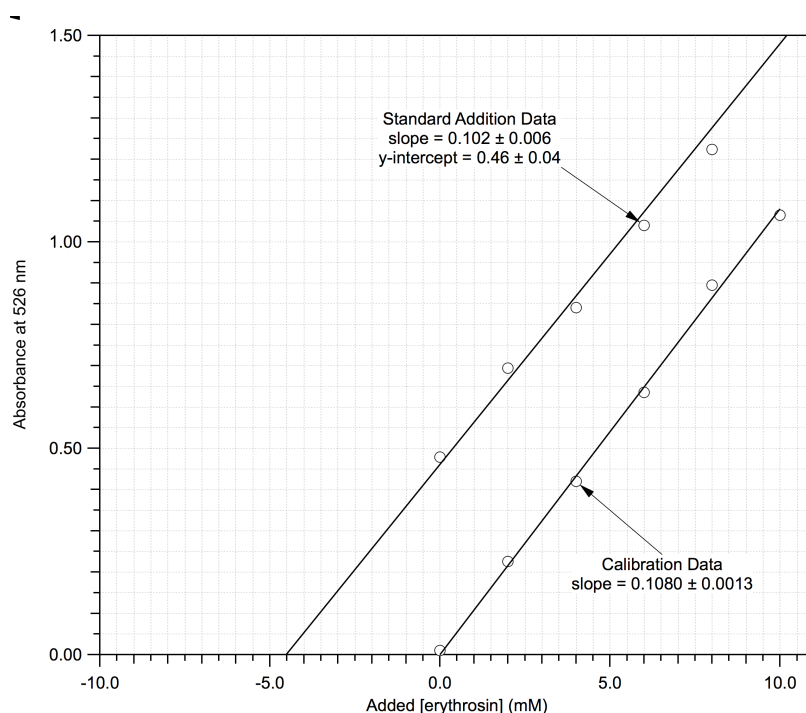


Figure 3. The standard addition and calibration curves of a hypothetical substance with a molar absorptivity of $1.080 \pm 0.0013 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$. The slope and y-intercept of the linear expressions fitted to both the experimental data sets are shown with their derived uncertainty expressed as one standard deviation. The graphical estimation of the substance's concentration of $4.5 \pm 0.1 \text{ mM}$.

Procedures

Record all measurements and data in the laboratory notebook. Estimate the uncertainty of each measurement made at the time the measurement is made.

Erythrosin Stock Solution: One or more solutions of approximately one millimolar (exactly known) erythrosin will be available in the laboratory. Do not contaminate them by inserting anything inside the bottle pour out a quantity into a flask. Roughly twenty milliliters of one of these solutions is sufficient to complete all of the measurements. Prepare 250

mL of a diluted erythrosin stock solution using a 50:1 dilution of one of the provided erythrosin solutions. Calculate the erythrosin concentration in this diluted solution.

Cuvette Standardization and Measurement Method: Set the spectrophotometer to measure absorbance at 526 nm. Obtain two cuvettes and label them with a marking pen clearly to differentiate between them but without writing on or obscuring the optical path. Fill both with distilled water. Estimate the volume of solution required to fill a cuvette for later reference. Zero the spectrophotometer with one of the labeled cuvettes. Measure the absorbance of the second cuvette. As the spectrophotometers are being shared it is necessary always to zero the instrument being used with distilled water in the first cuvette then measure the absorbance of the test solution in the second cuvette. Always correct the measured absorbance of the test solution in the second cuvette by the absorbance of water measured in the second cuvette. Do not waste time drying the second cuvette after each measurement. Rinse the test cuvette with tap water (no detergent is required) until all color is removed. Rinse the test cuvette briefly with distilled water. Shake the test cuvette to remove excess distilled water. Finally, rinse the cuvette twice or thrice with the solution to be measured.

Calibration Curve Method: Prepare a set of five solutions, each with a volume of fifty milliliters, with erythrosin concentrations of approximately but exactly known 2, 4, 6, 8 and 10 μM . There are several methods for quickly, conveniently and accurately preparing these solutions. One is to use a single 50-mL volumetric flask to prepare each solution one-at-a-time by pipetting the requisite volume of diluted erythrosin solution and making up the volume with distilled water. Alternately, a set of five Erlenmeyer flasks can be labeled and arrayed on the laboratory bench. The requisite volumes of diluted erythrosin solution are pipetted into each flask. The necessary volume of distilled water is then pipetted into each flask and the flasks carefully swirled to completely mix the contents. Alternately, the flasks can be capped with rubber stoppers (washed and paper towel dried) and shaken vigorously.

Measure the absorbance of each of the five solutions in the spectrophotometer.

Unknown Sample Solution: Weigh out approximately 50 mg (exactly measured) of erythrosin-sodium chloride solid mixture of unknown composition. Quantitatively transfer the solid sample to a 100-mL volumetric flask. Half-fill the flask with water and dissolve the solid completely. Finally fill to the mark with water and mix thoroughly.

Unknown Sample Measurement: Measure the absorbance of the unknown sample solution in the spectrophotometer. If the absorbance is outside of the range of the calibration curve solutions then dilute the unknown sample solution until a solution is obtained that displays an absorbance in the range of the calibration curve.

Standard Addition Method: Prepare a set of five solutions, each with a volume of fifty milliliters, with known erythrosin concentrations of approximately but exactly known 2, 4, 6, 8 and 10 μM but with one critical exception: add five (5) milliliters (or a different suitable volume if your earlier measurements indicate so) of the unknown sample solution to each solution before bringing it to volume.

Measure the absorbance of each of the five solutions in the spectrophotometer.

Results

Tabulate the calibration curve data. Prepare a figure containing a graph of the calibration curve data together with a linear least-squares curve fit of the experimental data to a linear equation consisting of slope. Report the uncertainty of the fitted slope on the figure. Estimate the erythrosin concentration in the test solution using both graphical and analytic methods. Calculate the composition of the unknown sample as weight fraction and weight percent erythrosin. Include an estimate of the uncertainty of this result using the calibration curve method.

Tabulate the standard addition data. Prepare a figure containing a graph of the calibration data and the standard addition data together with a linear least-squares curve fit of the standard addition experimental data to a linear equation consisting of slope and y-intercept. Report the uncertainty of the fitted slope and intercept on the figure. Be certain to plot the curve fit through the x-axis clearly showing this intersection. Estimate the erythrosin concentration in the test solution using both graphical and analytic methods. Calculate the composition of the unknown sample as weight fraction and weight percent erythrosin. Include an estimate of the uncertainty of this result using the standard addition method.

Perform an F-test of the slopes of the calibration and standard addition curves to identify the presence or absence of a matrix effect.

Discussion

Discuss your experimental results. Compare and contrast the determinations of the unknown sample composition by the two methods. Explain whether the data presented supports the presence or absence of a matrix effect for this system. Justify this with a chemically sound explanation.

References

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