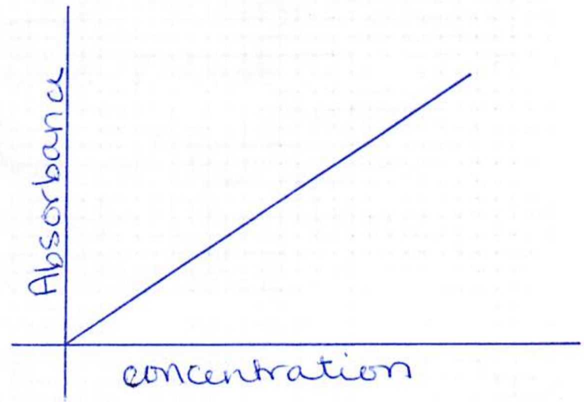
Calibration

Learning Objectives:

- Learn the relative merits of external calibration (by calibration curve or standard addition) and internal calibration.
- Identify and justify the best calibration method for a given application and be able to design a proper experiment.
- Be able to calculate the concentration of an unknown (and associated error) by each technique.

Calibration Curve

Draw an example graph of a calibration curve. Be sure to label the axis.



State the equation for the line of best fit.

$$\text{Absorbance} = m\,[\text{concentration}] + b$$

m: molar Absorbtivity · pathlength

b: Should be zero-ish

Describe the method of least squares analysis in words

the least squares method minimizes the vertical deviation to determine the line of best fit

Goodness of fit is frequently indicated using the R^2 value. What does this value mean?

R-squared is an indicator of the "goodness of fit" of the line of best fit to the data.

$$r^2 = \frac{[\Sigma(x_i - \bar{x})(y_i - \bar{y})]^2}{\Sigma(x_i - \bar{x})^2 \cdot \Sigma(y_i - \bar{y})^2}$$

State the equations needed to calculate the error associated with the concentration of an unknown.

$$S_x = \frac{S_y}{|m|}\sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(y - \bar{y})^2}{m^2\,(x_i - \bar{x})^2}}$$

y: measured absorbance of unknown

k: # unknown replicate measurements

n: # standards in cal curve

x_i: each individual

$$d_i = y_i - mx_i - b$$

$$S_y = \sqrt{\frac{\Sigma(d_i^2)}{n-2}}$$

State at least 5 requirements for a successful experiment using a calibration curve.

do not extrapolate beyond your standards range
use 6+ standards
do at least duplicate measurements of unknowns
Avoid serial dilutions that propagate dilution error
Measure standard solutions in a random order

4-30. Using the linear calibration curve in Figure 4-13, find the quantity of unknown protein that gives a measured absorbance of 0.264 when a blank has an absorbance of 0.095.

corrected absorbance = $0.264 - 0.095 = 0.169$ A

$A = 0.0163_0\,[\text{protein}] + 0.004_7$

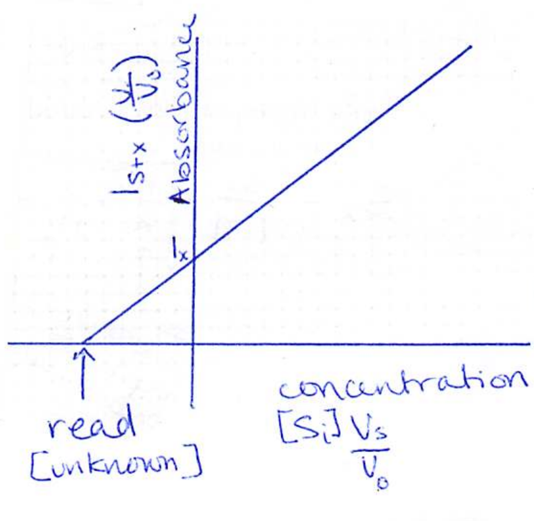
$\frac{0.169 - 0.004_7}{0.0163_0} = 10.079_7$ μg protein

Standard Addition

When is standard addtion the prefered method?

When the complex matrix of an unknown solution might contribute to the measured signal.

Draw an example graph of what data generated using each method would look like. Indicate on ~~each~~ the graph where you would meausre the concentration of an unknown. Be sure to label the axis.



State the equation of a standard addition line of best fit.

$$\underbrace{I_{s+x}\left(\frac{V}{V_0}\right)}_{y} = \underset{b}{\underset{\uparrow}{I_x}} + \underset{m}{\underset{\uparrow}{\frac{I_x}{[X_i]}}}\underbrace{[S_i]\left(\frac{V_s}{V_0}\right)}_{x}$$

State the equations needed to calculate the error associated with the concentration of an unknown.

$$S_x = \frac{S_y}{|m|}\sqrt{\frac{1}{n} + \frac{\bar{y}^2}{m^2 \Sigma(x_i - \bar{x})^2}} \qquad S_y = \sqrt{\frac{\Sigma(d_i^2)}{n-2}}$$

Why is it desireable to the method of standard addition to add a small volume of concentrated standard rather than a large volume of dilute standard?

so that the matrix is similar or nearly the same for all solutions measured

5-24 An unknown sample of Cu^{2+} gave an absorbance of 0.262 in an atomic absorption analysis. Then 1.00 mL of solution containing 100.0 ppm (= µg/mL) Cu^{2+} was mixed with 95.0 mL of unknown, and the mixture was diluted to 100.0 mL in a volumetric flask. The absorbance of the new solution was 0.500.

(a) Denoting the initial, unknown concentration as $[Cu^{2+}]_i$, write an expression for the final concentration, $[Cu^{2+}]_f$, after dilution. Units of concentration are ppm.

(b) In a similar manner, write the final concentration of added standard Cu^{2+}, designated as $[S]_f$.

(c) Find $[Cu^{2+}]_i$ in the unknown.

a $M_1V_1 = M_2V_2$ $\qquad [Cu_f] = \frac{95\text{ mL}}{100\text{ mL}}[Cu_i]$

b $[Cu_f] = \frac{1\text{ mL}}{100\text{ mL}}[S_f] = 1\text{ ppm } Cu^{2+}$

c $[Cu_f] = 0.95\,[Cu_i] + 1\text{ ppm } Cu^{2+}$

$$\frac{[Cu_i]}{0.95[Cu_i] + 1\text{ ppm } Cu^{2+}} = \frac{0.262}{0.500}$$

$$0.500\,[Cu_i] = 0.2489\,[Cu_i] + 0.262\text{ ppm } Cu^{2+}$$

$$0.252\,[Cu_i] = 0.262\text{ ppm } Cu^{2+}$$

$$[Cu_i] = 1.039\text{ ppm} \Rightarrow 1.04\text{ ppm } Cu^{2+}$$

Internal Standards

State when standard additions and internal standards, instead of a calibration curve, are desirable and why.

simple systems allow for the use of calibration curves. Internal standards are generally used to account for small changes in the ~~method~~ ~~of~~ sample prep or instrumental method (ie chromatography with small changes in retention times.) That need to be accounted for.

Standard addition is required in complex matricies that interfere w/ the measurement of the analyte.

When preparing calibration standards for each of the following methods, which of the following are held constant?

1. Calibration Curve____________

2. Standard Addition B, A

3. Internal Standard A, C

A. final amount
B. amount of sample
C. amount of standard
D. amount diluent added

5-30. A solution containing 3.47 mM X (analyte) and 1.72 mM S (standard) gave peak areas of 3 473 and 10 222, respectively, in a chromatographic analysis. Then 1.00 mL of 8.47 mM S was added to 5.00 mL of unknown X, and the mixture was diluted to 10.0 mL. This solution gave peak areas of 5 428 and 4 431 for X and S, respectively.

(a) Calculate the response factor for the analyte.

(b) Find the concentration of S (mM) in the 10.0 mL of mixed solution.

(c) Find the concentration of X (mM) in the 10.0 mL of mixed solution.

(d) Find the concentration of X in the original unknown.

	[ ]	A
X	3.47 mM	3473
S	1.72 mM	10.222

a $F = \dfrac{\dfrac{A_x}{[X]}}{\dfrac{A_s}{[S]}} = \dfrac{\left(\dfrac{3473}{3.47}\right)}{\left(\dfrac{10.222}{1.72}\right)} = \dfrac{1000.864}{5943.02} = 0.1684$

b $M_1V_1 = M_2V_2$

$8.47\text{ mM} \cdot 1\text{ mL} = X \cdot 10\text{ ml}$ $X = 0.847\text{ mM}$

c $\dfrac{5428}{[X]} = \underbrace{0.1684 \cdot \dfrac{4431}{0.847\text{mM}}}_{880.968}$

$[X] = 6.161\text{ mM}$

d $M_1V_1 = M_2V_2$

$6.161\text{ mM} \cdot 10\text{mL} = 5\text{mL}\ X$ $X = 12.322\text{ mM}$