Chapter 5  Calibration Methods

Homework:

Chapt: 5:  5-7, 5-16, 5-17, 5-21, 5-22, 5-23

Due Friday, February 3
Chapter 5  Calibration Methods

*Calibration involves developing a functional relationship between signal(s) and concentration(s) of analyte(s)*

- **Signal**: response of system (absorbance, current, intensity, ion count, peak area, etc.)
  This is the dependent or \( y \) variable

- **Concentration**: known concentration(s) of analyte(s) in standards.
  This/these are the independent or \( x \) variable(s)
Least squares method web site:
http://standards.nctm.org/document/eexamples/chap7/7.4/#applet

Simplest calibration is **linear relationship**: signal vs. concentration

Recall: Straight line equation -  \( y = mx + b \)

**Least squares method** is a technique for finding the best line through a given set of points. The technique is based on minimizing the square of the deviations from each point and the line. See Harris pg. 81 – 84 for how to calculate least squares fit.

Using 1\(^{st}\) and 2\(^{nd}\) points:
\[ m = \frac{\Delta y}{\Delta x} \]
\[ = \frac{0.834}{2.917} \]
\[ = 0.281 \]

\( m \) of line = 0.2564

*Slight difference, points not perfectly linear.*
How do you know if you have a “good” line?  
(This is not covered in Harris, but is necessary to know!!!)

**Correlation coefficient**: Deviation of the each point from the calculated straight line, listed as an “r” value.

**Coefficient of Determination**: Square of correlation coefficient – this is the value of most importance. Describes the “straightness of the line. Also referred to as the “r^2” value.

- $r^2$ values are from 0 to 1 – the closer the value is to 1, the better the fit of the line.
- The $r^2$ value can be readily determined with a spreadsheet, and provided when plotting with a treadline (linear regression).
How tall is a 10 yr old boy?
From growth charts at 50%, 10 yr old boy is 4.6 ft tall.
This value does not fall on calibration line. WHY???
To be useful, a calibration curve must:

• Have a defined relationship a measurable quantity (usually concentration) and the signal measure.
  • Often this relationship is linear, but other relationships can also be useful.
• Be collected under the same, or near same, conditions as what exists for the analyte.
  • Similar matrix
  • Similar species
  • Similar experimental conditions (sample holder, lab temperature, instrument settings, etc.)
• A minimum of 3 data points over a range of concentrations should be collected to define the line (or other calibration relationship). The concentration of the analyte must lie within the calibration range.
An example of a calibration model

![Graph showing the relationship between ions/sec and I- concentration (ppb). The x-axis represents the I- concentration in ppb, ranging from 0.001 to 100, and the y-axis represents ions/sec, ranging from 1 to 10,000,000. The data points form a linear trend.]
A few things to remember on linear regression

• One assumes errors in X are negligible
• One assumes that the correct model is picked
• Evaluate using standard errors of slope, intercept
• Examine “residuals” for funny behavior
• Magnitude of r, r² should be examined
Two reasons why the line is bad: lack of fit, and purely experimental uncertainty.

- Purely experimental uncertainty: the "noisy line"
- "Lack of fit": systematic deviation from the chosen model
How to design the calibration set

• # settings for $X > # parameters in model will allow “lack of fit” to be examined
  (For example, linear fit: need at least 3 points)
• Replicate measurements of $Y$ for a given setting of $X$ will allow “purely experimental uncertainty” to be examined
The diagram shows a plot of \( \ln k_{11} \) against \( 1/T \). The data points are scattered across the graph, indicating a relationship between the natural logarithm of the reaction rate constant \( k_{11} \) and the reciprocal of temperature \( 1/T \). The values of \( k_{11} \) range from approximately 0.0033 to 0.0037, and the corresponding temperatures \( 1/T \) range from 0.0033 to 0.0037.
Method of standard additions

- **Known amounts of analyte are added to aliquots of sample**
- Signals are measured as a function of concentration added
- Modeling process: $\text{Signal} = k \cdot C_{\text{added}} + b$
- Equation is solved for concentration where $\text{Signal} = 0$ (an extrapolation process)
Equations for method of standard addition

Analyte only:
\[ I_x = k \ [X_i] \]

The signal intensity is proportional to the concentration of the analyte.

Analyte + Standard:
\[ I_{S+X} = k([S_f] + [X]_f) \]

The signal intensity is proportional to the concentration of the analyte and the standard.

So….
\[ \frac{I_x}{I_{S+X}} = k \left( \frac{[X_i]}{k([S_f] + [X]_f)} \right) = \frac{[X_i]}{k([S_f] + [X]_f)} \]

To get \(X_f\) and \(S_f\): MUST KNOW DILUTION FACTORS.

\[ [X_f] = [X_i] \left( \frac{V_i}{V_f} \right) \quad \text{and} \quad [S_f] = [S_i] \left( \frac{V_i}{V_f} \right) \]
Example of Standard Addition Plot

Y

X

original concentration

CMH 320  Chapt 5  Lecture 6
Why is standard addition used?

• Std. Add. is effective at correction of “matrix effects”, i.e., the sensitivity (signal/conc) is sample-dependent.

• Std. Add. will NOT correct for additive interferences, i.e. if another species is present that contributes signal at the sensor being measured.

• Std. Add. is standard practice in some techniques.
Internal standardization

• A substance known as an “internal standard” is added to samples and standards
• Used to correct for drift (changes in sensitivity over time) and matrix effects (sample-related changes in sensitivity)
• Effective if certain requirements can be met; less work than std. add.
**Internal Standard Equation (linear)**

\[
\frac{I_{\text{analyte}}}{I_{\text{intstd}}} = k*[\text{Analyte}] / [\text{internal std}]
\]

**Requirements for an internal standard**

- Technique must be multicomponent - must separately measure signals for analyte and internal standard
- No interferences: analyte\(\iff\) internal std or sample matrix \(\iff\) internal std
- Internal standard must emulate drift and matrix effect behavior
- Internal standard is not native in the sample
What is a blank?

- **A calibration blank** is used to determine the response (signal) given by the measurement system in the absence of any added analyte.

- **Method/preparation/reagent/procedural blank** is used to determine the amount of analyte added to the system as a result of the preparation.

- The blank can either be subtracted from analyte signal, or used as zero point in calibration.