Chem 321 Lecture 6 - Calibration Methods

9/12/13

Student Learning Objectives

Calibration Methods

Most analytical methods rely on a standard. Such an approach is sometimes referred to as a **comparator method**. In such a procedure, a carefully prepared standard is run through the same analysis procedure used for the unknown samples and the results (signals) from the standard and unknown are compared. When many standards are run, a **calibration curve** is usually made in which the signal from the standard analysis is plotted against the standard concentration. Often the relationship between the signal and concentration is linear (i.e., the signal is directly proportional to concentration) over some range of concentrations and a **linear least-squares line** is used to fit the data. In a least-squares fit, a line is drawn through the plotted data points so that the distance between each data point and the line is minimized. The most common form of least-squares analysis minimizes the distance between the data points and the line along the *y*-axis. This assumes that the values for the *x*-coordinates generally have much less error associated with them than do the *y*-coordinate values.

The straight line through the data will take the form of $y = mx + b$, where m is the **slope** of the line and b is the ***y*-intercept**. Knowledge of m and b for the calibration curve allows one to calculate the sample concentration (x) from the sample signal (y). A good summary of the mathematics behind the linear least=squares fitting is given in **Appendix B** of the lab manual. A discussion of how to use Excel to plot and to fit data is provided in **Appendix A** of the lab manual.

Check for Understanding 3.1 **Solution**

1. Data are fit with a least squares straight line that has a slope of -1.29872 x 10^4 ± 1.3190 x 10^3 and *y*-intercept of 256.695 ± 32.357. Express the slope and intercept and their uncertainties with an appropriate number of significant figures.

Results obtained from a calibration curve are most reliable when interpolations are done. This is the case when the signals from the unknown samples fall between the highest and lowest signal from the standards. Samples may need to be diluted in order to achieve this.

At some standard concentration the signal may no longer be directly proportional to concentration and the calibration curve becomes non-linear. In this case, the data points must be fit with another mathematical form, often a quadratic equation ($y = ax^2 + bx + c$). It is perfectly legitimate to use a non-linear calibration curve, however, one must assume that whatever effect is causing the non-linear response in the standard signal is also at work in the unknown samples.

For some measurements it is not possible to adequately control the quantity of sample analyzed or the response of the instrument. Your measurements for the gas chromatography experiment in lab are examples of this situation because you are not able to inject the same amount of sample in each run. In order to make quantitative measurements under these conditions, one can use an **internal standard**. An internal standard is a substance not normally present in an unknown sample. It must have a readily measured signal during analysis and it must not interfere with the analyte signal. Typically, it is added to each unknown sample and standard so that its concentration is the same in all samples. Then both the standards and unknowns are analyzed in the same fashion. Each signal for the analyte is **normalized** by dividing the analyte signal by the signal for the internal standard. A calibration curve is then made by plotting the normalized standard signal versus standard concentration. This approach assumes that there is a linear response for both the analyte and the internal standard.

In the gas chromatography experiment, you will add decane, a component not present in your unknowns or standards, to each sample to the same concentration level. When you analyze the samples you will get a signal (peak area) for decane and for each component of interest. The size of the decane peak area is proportional to how much sample you analyze (this varies from run to run). To account for differences in the amount of sample analyzed, you divide the peak area of a component by the decane area before comparing the signals found in a standard and unknown.

When the sample being analyzed is a rather complex matrix, other components present in the sample can affect the analyte signal (the **matrix effect**). A direct comparison to standards is not valid because it is usually impossible to duplicate the sample matrix when preparing the standards. In such situations the **method of standard addition** can be used. The underlying assumption is that the analyte signal (I) is directly proportional to the analyte concentration ([A]). In other words, $I = k[A]$.

One way to apply this method is to prepare a series of samples of constant volume, each containing the same amount of the unknown with varying amounts of added analyte standard. Then all samples are analyzed and the analyte signal is plotted versus the concentration of the added analyte in the sample. Such a plot is

shown below. The *x*-intercept of the linear least-squares fit to the data is the negative of the concentration of the analyte in the diluted unknown. The *x*-intercept can be calculated from the equation for the linear least-squares fit (y = mx + b) for *y* = 0.

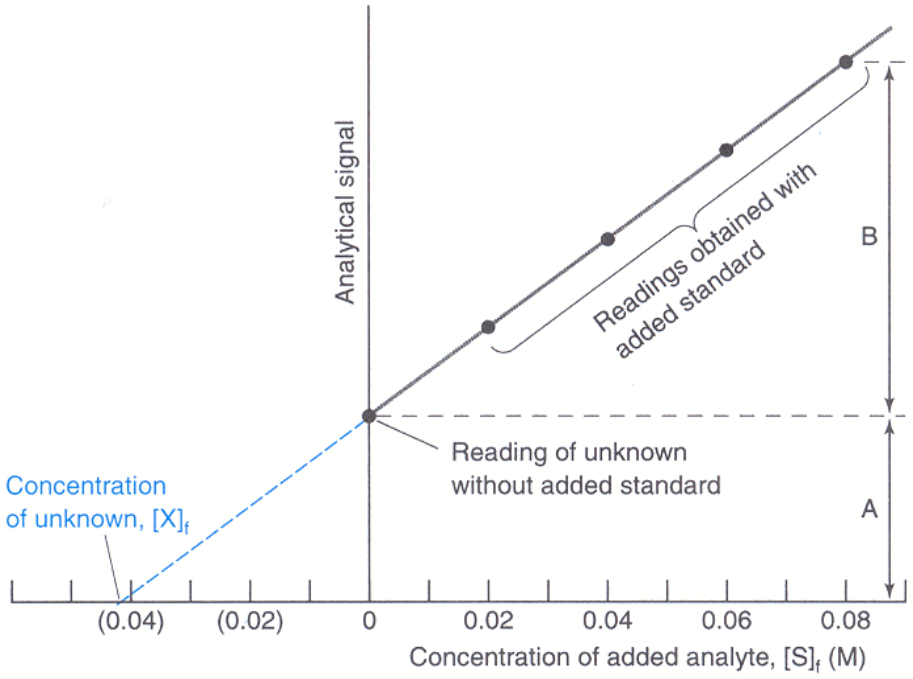


Figure 3.1 Standard addition calibration curve

In order to see how this result is obtained, recall that for the unknown with no added analyte $I_x = k[X]$ where I_x is the analyte signal for the unknown, $[X]$ is the analyte concentration in the unknown and k is a proportionality constant. When analyte standard is added the overall signal can be expressed as $I_{x+s} = k[X] + k[S]$ where $[S]$ is the concentration of the added analyte in the diluted sample. Dividing these two equations yields:

$$\frac{I_x}{I_{x+s}} = \frac{k[X]}{k[X] + k[S]}$$

Canceling the common term *k* and solving for *[X]* gives

$$[X] = \frac{I_x[S]}{I_{x+s} - I_x}$$

At the *x*-intercept, $I_{x+s} = 0$ and *[X]* = *-[S]*. In order to minimize the error associated with extrapolation of the linear least-squares line to the *x*-axis, the amount of added analyte should increase the analyte signal by approximately a factor of two. This method of standard addition will be used in the determination of fluoride experiment.

Propagation of Uncertainty with a Calibration Curve

The uncertainty in quantity *x* (s_x) calculated from a linear least-squares line (y = mx + b) is given by:

$$s_x = \frac{s_y}{|m|}\sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(y - \bar{y})^2}{m^2 \Sigma (x_i - \bar{x})^2}}$$

where $s_y = \sqrt{\frac{\Sigma(y_i - mx_i - b)^2}{n - 2}}$

m is the slope of the line

k is the number of measurements of the unknown

n is the number of data points for the calibration curve

y is the average *y* value for k measurements of the unknown

$\bar{y}$ is the mean value of *y* for the points on the calibration curve

x_i are the individual values of *x* for the points on the calibration curve

$\bar{x}$ is the mean value of *x* for the points on the calibration curve

The confidence interval for x is then given by $\pm ts_x$ where t corresponds to the value for n - 2 degrees of freedom. This rather daunting formula for s_x is rather easily evaluated using Excel and the LINEST function. In Excel the output of the LINEST function includes the m, b and s_y parameters needed in the calculation of s_x. The other quantities can be quickly evaluated once the calibration data are entered. Example 3.1 illustrates how all of these calculations can be done in Excel.

Example 3.1

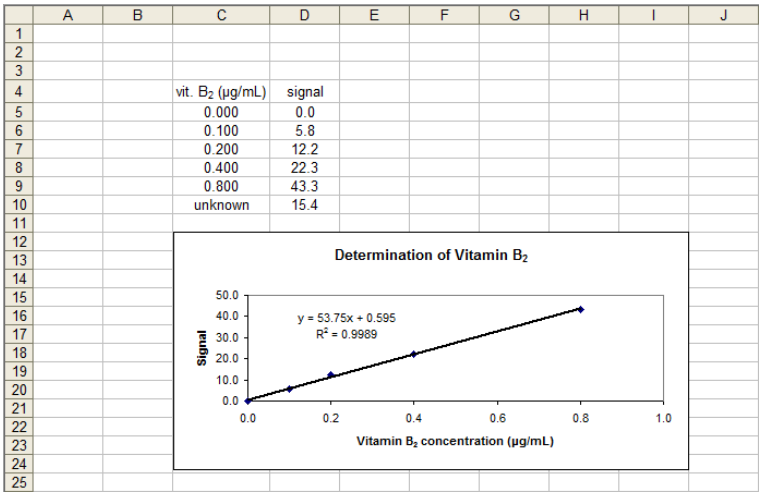
Problem

Determine the uncertainty in the vitamin B_2 concentration of an unknown based on the data below and find the 95% confidence interval for this measured value.

Vitamin B_2 Concentration (µg/mL)	Signal intensity (arbitrary units)
0.000	0.0
0.100	5.8
0.200	12.2
0.400	22.3
0.800	43.3
unknown	15.4

Solution

First, enter the data into the cells in an Excel spreadsheet as shown below and prepare a calibration curve with a linear least-squares line (trendline).



Example 3.1 (continued)

Solution

To use the LINEST function in Excel, follow these steps.

1) Highlight cells G6:H8

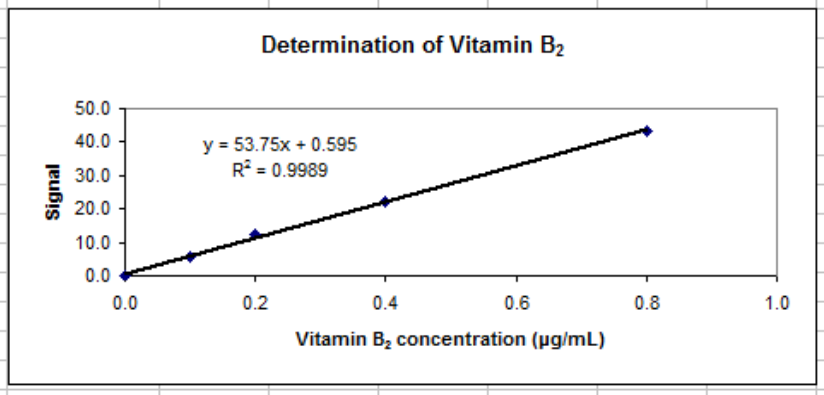
2) Type =LINEST(D5:D9,C5:C9,TRUE,TRUE)

3) For PC, press CTRL+SHIFT+ENTER

   For Mac, press COMMAND+RETURN

The LINEST output should look like the following. The bolded text entries below indicate what each item corresponds to.

	C	D	F	G	H	I
4	vit. B_2 (µg/mL)	signal				
5	0.000	0.0		LINEST output		
6	0.100	5.8	**m**	53.75	0.595	**b**
7	0.200	12.2	**s_m**	1.017759	0.419633	**s_b**
8	0.400	22.3	**R^2**	0.998926	0.643687	**s_y**
9	0.800	43.3				
10	unknown	15.4				

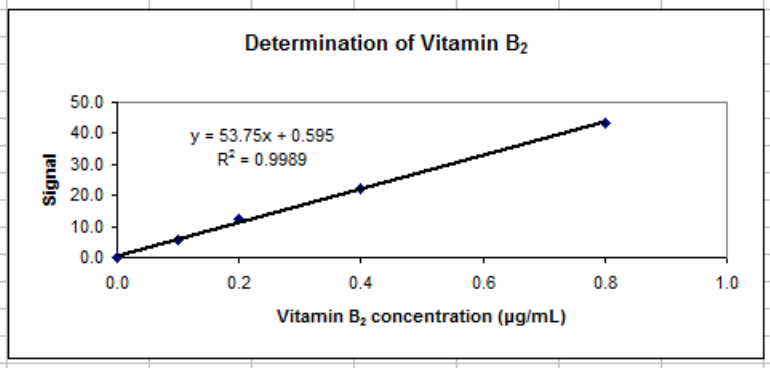


Example 3.1 (continued)

Solution

The other quantities needed to calculate s_x can now be evaluated using the information entered in the spreadsheet. The formula used in each case is given to the right of the cell containing the quantity.

	A	B	C	D	E	F	G	H	I	J
1										
2										
3										
4			vit. B_2 (µg/mL)	signal						
5			0.000	0.0			LINEST output			
6			0.100	5.8		m	53.75	0.595	b	
7			0.200	12.2		s_m	1.017759	0.419633	s_b	
8			0.400	22.3		R^2	0.998926	0.643687	s_y	
9			0.800	43.3						
10			unknown	15.4						
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21										
22										
23										
24										
25										
26			n =	5	D26=COUNT(C5:C9)					
27			Mean y =	16.7	D27=AVERAGE(D5:D9)					
28			$\Sigma(x_i$ - mean $x)^2$	0.4	D28=DEVSQ(C5:C9)					
29										
30			Measured y =	15.4	D30=D10					
31			k =	1	D31=COUNT(D10)					
32			Calculated x =	0.275442	D32=(D10-H6)/G6					
33										
34			s_x =	0.013127	D34=(H8/G6)*SQRT((1/D31)+(1/D26)+((D30-D27)^2/(G6^2*D28)))					
35										



The measured concentration and uncertainty in the unknown vitamin B_2 is 0.27 ± 0.01_3 µg/mL. The 95% confidence interval is

$$0.27 \text{ µg/mL} \pm ts_x = 0.27 \text{ µg/mL} \pm (3.182)(0.01_3 \text{ µg/mL}) = 0.27 \pm 0.04 \text{ µg/mL}$$