Linear Least Squares Fit of Standard Curves

$$y = mx + b$$

$$\bar{x} = \frac{1}{N}\sum x_i \; ; \; \bar{y} = \frac{1}{N}\sum y_i$$

$$S_{xx} = \sum (x_i - \bar{x})^2 = \sum x_i^{\,2} - \frac{(\sum x_i)^2}{N}$$

$$S_{yy} = \sum (y_i - \bar{y})^2 = \sum y_i^{\,2} - \frac{(\sum y_i)^2}{N}$$

$$S_{xy} = \sum (x_i - \bar{x})(y_i - \bar{y}) = \sum x_i y_i - \frac{\sum x_i \sum y_i}{N}$$

All summations run from $i = 1$ to N.

Slope: m

$$m = \frac{S_{xy}}{S_{xx}}$$

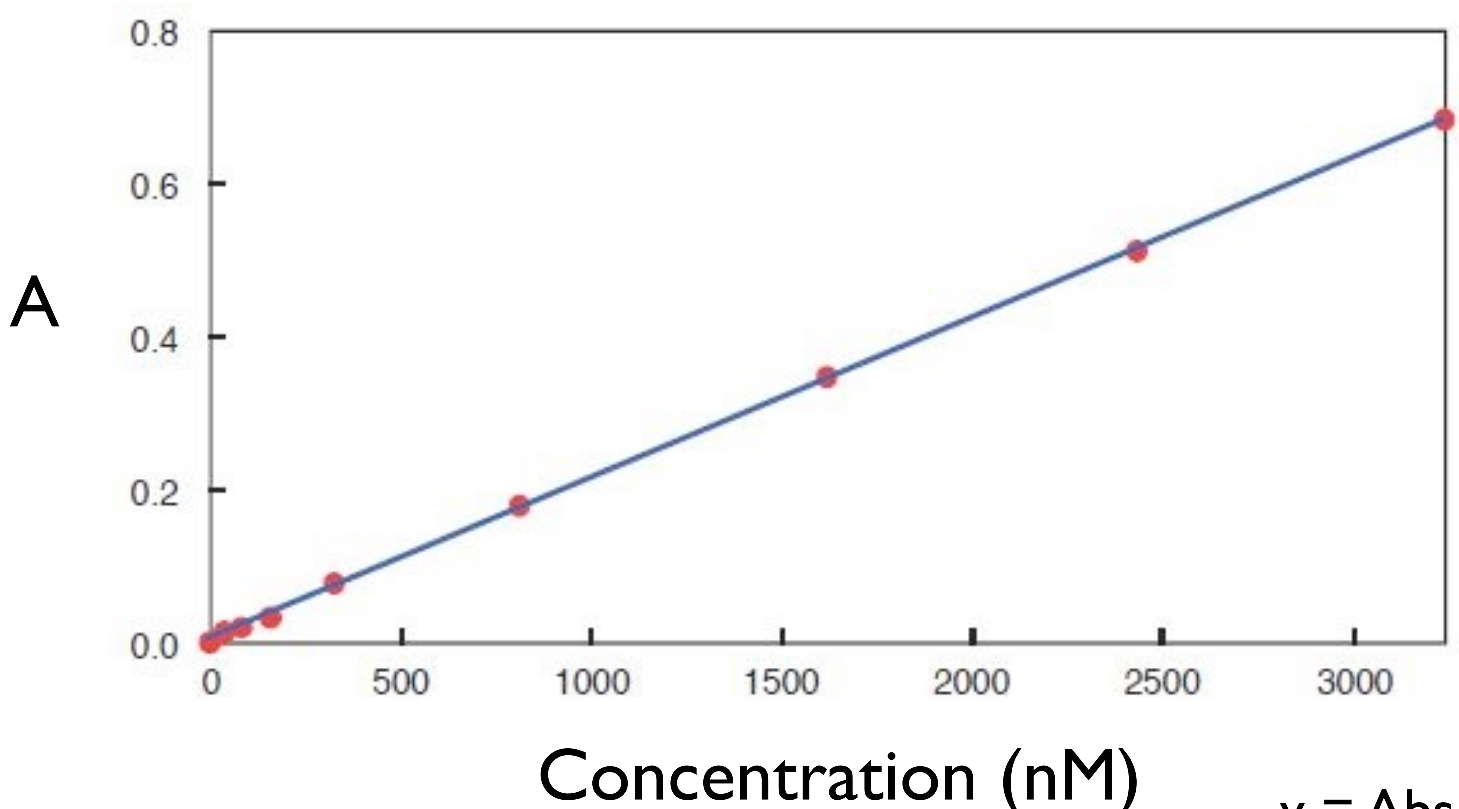
Intercept: b

$$b = \bar{y} - m\bar{x}$$

R. Corn - Chem M3LC
UC Irvine

We can measure the concentration of a species by measuring the Absorbance at a particular wavelength.

Beer's Law: $A = \epsilon d C$



y = Absorbance
m = εd
x = Concentration

Linear Calibration Curve: $y = mx + b$

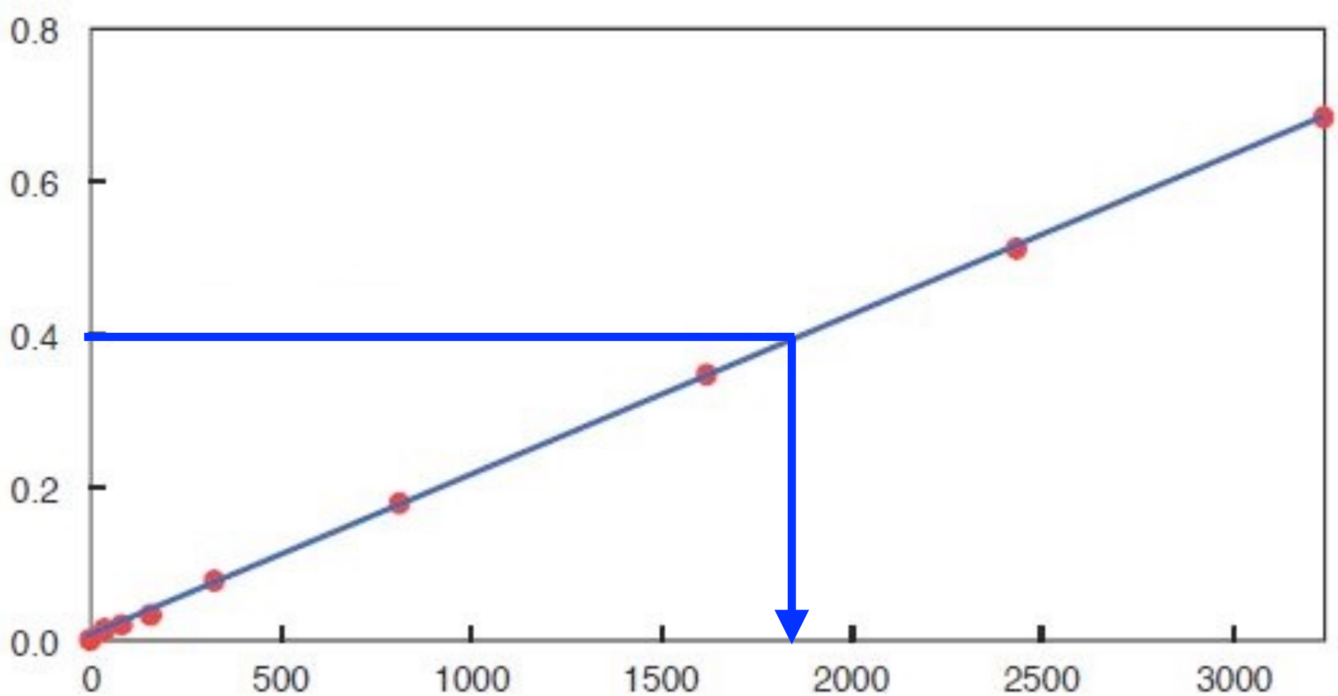
b = *value of* y *at* $x=0$.

Using the Standard Curve with Unknowns

Just measure the Absorbance, and calculate the Concentration!

Beer's Law: $A = \epsilon d C$

Abs



Concentration (nM)

Concentration = (y-b)/m = 1800 nM

We need to calculate m and b first!

Equations for Fitting a Linear Calibration Curve ($y = mx + b$) from a set of N (x,y) data points:

$$y = mx + b$$

$$\bar{x} = \frac{1}{N}\sum x_i \; ; \; \bar{y} = \frac{1}{N}\sum y_i$$

$$S_{xx} = \sum (x_i - \bar{x})^2 = \sum x_i^2 - \frac{(\sum x_i)^2}{N}$$

$$S_{yy} = \sum (y_i - \bar{y})^2 = \sum y_i^2 - \frac{(\sum y_i)^2}{N}$$

$$S_{xy} = \sum (x_i - \bar{x})(y_i - \bar{y}) = \sum x_i y_i - \frac{\sum x_i \sum y_i}{N}$$

All summations run from $i = 1$ to N.

Slope: m

$$m = \frac{S_{xy}}{S_{xx}}$$

*Intercept: b

$$b = \bar{y} - m\bar{x}$$

m and b can be calculated from this set of 5 summations

*note: the intercept b is due to background

We can also calculate the slope and intercept standard deviations from summations:

Regression Standard Deviation: s_r

$$s_r = \sqrt{\frac{S_{yy} - m^2 S_{xx}}{N-2}}$$

s_r is used to calculate s_m and s_b

Slope Standard Deviation: s_m

$$s_m = \sqrt{\frac{{s_r}^2}{S_{xx}}}$$

s_m uses s_r and S_{xx}

Intercept Standard Deviation: s_b

$$s_b = s_r \sqrt{\frac{\sum {x_i}^2}{N \sum {x_i}^2 - (\sum x_i)^2}}$$

s_b uses s_r and three other simple summations

We also can calculate 95% confidence intervals for the slope and intercept from the standard deviations:

Error Analyis Equations for a Linear Calibration Curve:

95% confidence level for the slope:

$$m \pm t_{N-2} s_m$$

95% confidence level for the intercept:

$$b \pm t_{N-2} s_b$$

where $t_{N\text{-}2}$ is the Student T-factor for N-2 degrees of freedom.

Note that the d.o.f. is N-2 (we calculate slope AND intercept)

Finally, here are the (somewhat complex) equations for calculating the standard deviation and confidence interval when using the calibration curve.

The standard deviation for results obtained from the calibration curve is s_C:

$$s_c = \frac{s_r}{m}\sqrt{\frac{1}{C} + \frac{1}{N} + \frac{(y_c - \bar{y})^2}{m^2 S_{xx}}}$$

This equation is used to calculate the standard deviation s_C for an average value x_C obtained from of a set of C replicate measurements of an unknown with a mean y_C:

$$x_c = \frac{y_c - b}{m}$$

when the calibration curve contains N points. As with the slope and the intercept, the 95% confidence level for this average is:

$$x_c \pm t_{N-2} s_c$$

where $t_{N\text{-}2}$ is the Student T-factor for N-2 degrees of freedom.

Linear Least Squares Fit of Standard Curves

B8 fx =C6^2

	A	B	C	D	E	F	H	I	K	L
1	Standard #	x: Conc. (mM)	y: Abs	xi^2	yi^2	xi*yi	N	4	Unknown	
2	1	0.008	0.223	0.000064	0.049729	0.001784	xbar	0.022	Trial 1	0.705
3	2	0.016	0.448	0.000256	0.200704	0.007168	ybar	0.59825	Trial 2	0.711
4	3	0.024	0.67	0.000576	0.4489	0.01608	Sxx	0.00056	yc (average)	0.708
5	4	0.04	1.052	0.0016	1.106704	0.04208	Syy	0.37442475	C	2
6	SUM	0.088	2.393	0.002496	1.806037	0.067112	Sxy	0.014466		
7	(SUM xi)^2	0.007744							xc	0.02624858
8	(SUM yi)^2	5.726449					m	25.8321429	sc	0.00065723
9							b	0.02994286		
11							sr	0.01919598		
12							sm	0.81117828		
13							sb	0.02026323		
15							t (N-2)	4.303		
17							95%CI			
18							slope (m)	3.49050014		
19							Intercept (b)	0.08719267		
20							unknown (x)	0.00282806		

We will create a spreadsheet to make these linear least squares fit calculations!