Biological Chemistry Laboratory
Biology 3515/Chemistry 3515
Spring 2018

Lecture 6

Curve Fitting, Part I

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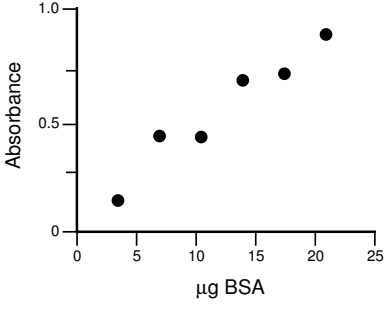
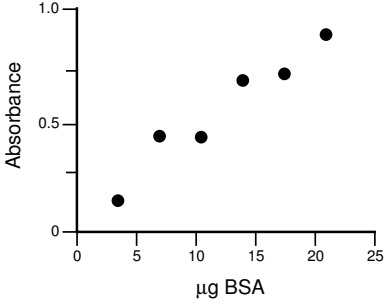
The Curve-Fitting Problem



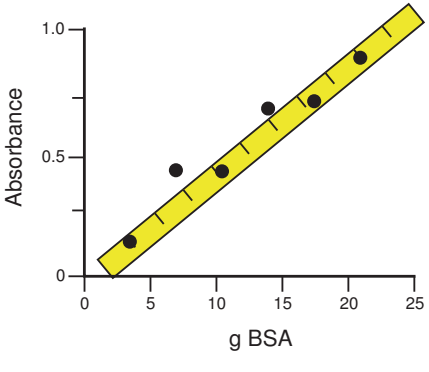
- How do we find the equation of the line (or other function) that best "fits" the experimental data?
- What assumptions do we make when fitting data to a function?
- Reminder: Graph axes should be clearly labeled and specify the correct units!

The Curve-Fitting Problem



- Two general kinds of situations:
  - We have a theoretical model that predicts a particular function, and we want to both test the model and estimate parameters that define the model.
  - We don't have a particular model in mind, but there is an empirical relationship that fits the data, and we want to estimate the parameters.
- Which category does the Bradford calibration fall into?

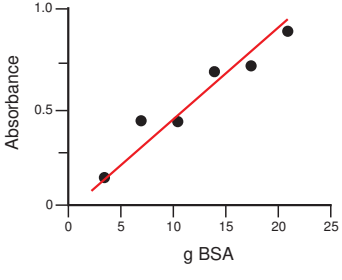
The Ruler Method



- Advantages?
  - It's easy and inexpensive!
- Disadvantages?
  - It's subjective. Different individuals will get different results.
    The more scatter, the bigger the problem!
  - Pretty much limited to the straight-line function.
  - Doesn't provide a measure of how well the data fit the function.

Clicker Question #1

Which is the “best” fit?



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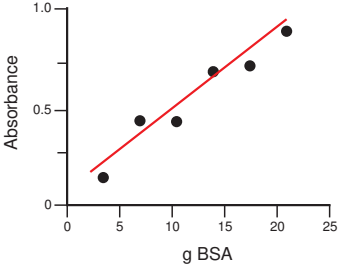

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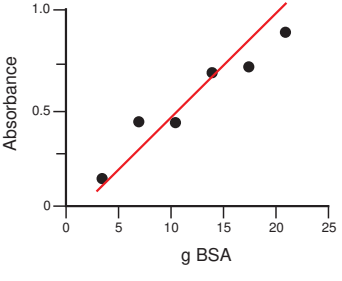

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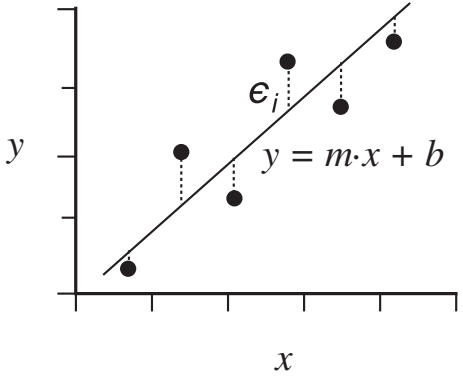
All answers count (for now)!

The Method of Least Squares



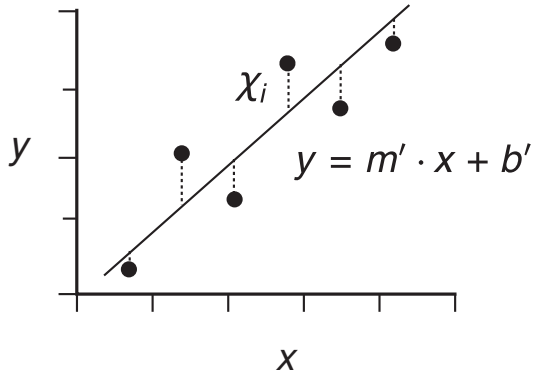
- Key Assumption: The experimental values of y are determined by a linear (or other) function of x *and* random error in the measurements.

$$y_i = m \cdot x_i + b + \epsilon_i$$

m and b are the "true" values of the parameters, and ϵ_i is the error in each measurement.

Chose an Arbitrary Line Given by the Equation:

$$y = m' \cdot x + b'$$

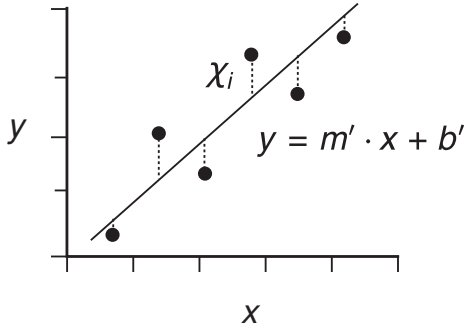


- The observed values of y are then described by the equation:

$$y_i = m' \cdot x + b' + \chi_i$$

- χ_i is referred to as the "residual" for each point, and is distinct from the error, ϵ_i. (We never really know the values of ϵ_i.)
- We want to choose m' and b' so that they represent the best estimates

χ^2: The Sum of the Squares of the Residuals

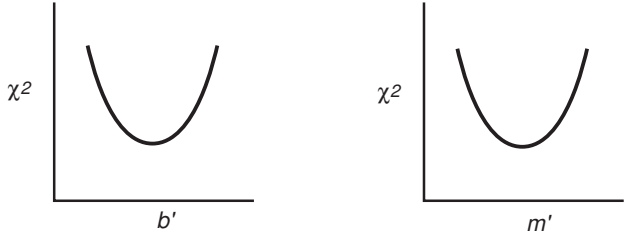


$$\chi^2 = \sum \chi_i^2$$
$$= \sum (y_i - (m' \cdot x_i + b'))^2$$

- Adjust m' and b' to minimize the value of χ^2 for the particular values of x_i and y_i in the experimental data set.
- Why are the residuals squared?

Minimization of χ^2

- χ^2 is a function of both b' and m'



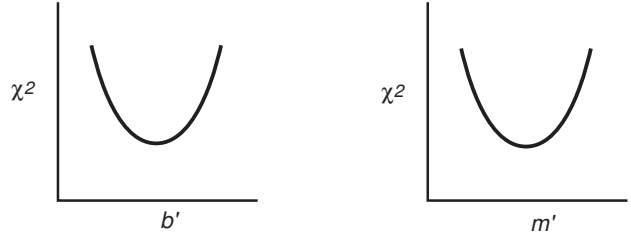
- How do we find values of b' and m' that minimize χ^2?
- Take derivatives of χ^2 with respect to b' and m' and set them to zero.

$$\frac{d}{db'} \sum \left(y_i - (m' \cdot x_i + b')\right)^2 = 0$$

$$\frac{d}{dm'} \sum \left(y_i - (m' \cdot x_i + b')\right)^2 = 0$$

Two equations, with unknowns b' and m'.

Minimization of χ^2



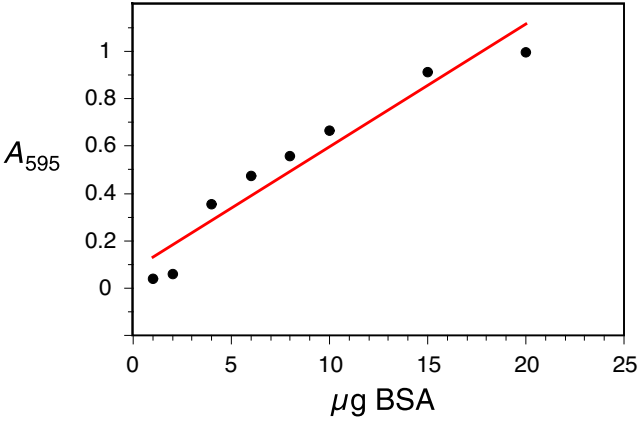
- For a linear function, there is a (relatively) simple solution to the two equations:

$$b' = \frac{\sum x_i^2 \sum y_i^2 - \sum x_i \sum x_i y_i}{N \sum x_i^2 - \left(\sum x_i\right)^2}$$

$$m' = \frac{N \sum x_i y_i - \sum x_i \sum y_i}{N \sum x_i^2 - \left(\sum x_i\right)^2}$$

N is the number of experimental x, y pairs.

A Linear Least-squares Fit to Bradford Calibration Data



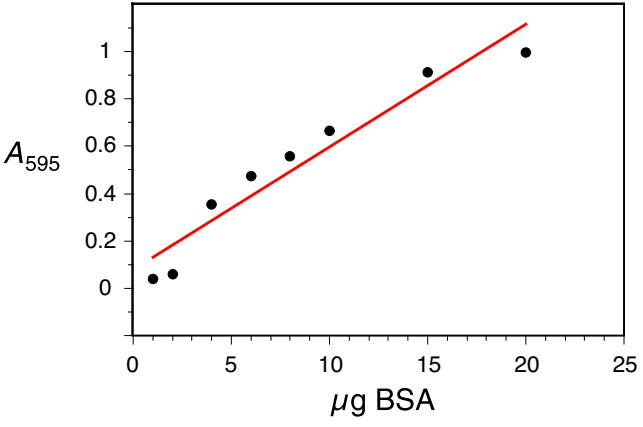
- The estimated parameters for $y = mx + b$:

  $m = 0.052 \pm 0.006$

  $b = 0.08 \pm 0.06$

- The uncertainties are analogous to the standard error of the mean.
- What are the units for the parameters?
- How “good” is the fit?

How Do We Judge the Goodness of Fit?

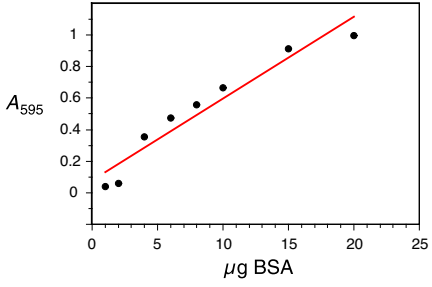


- The final, minimized, value of χ^2. For this fit:

$$\chi^2 = 0.062$$

- Can use this value to compare this fit to fits of the same data to other functions.
- But, actual value does not have a clear meaning.
- Adding more measurements will almost always increase χ^2.

The Coefficient of Determination, R^2



- What fraction of the total variation of y-values is accounted for by the fit function?
- For this fit:

$$R^2 = 0.93$$

Compare χ^2 to the total variation in y-values.

- Define the total sum of squares:

$$SS_{\text{tot}} = \sum (y_i - \bar{y})^2$$

$\bar{y}$ = mean of y-values.

- The ratio:

$$\frac{\chi^2}{SS_{\text{tot}}}$$

represents the fraction of the variation that is *not* accounted for by the fit function.

- The fraction of variation that *is* accounted for the function:

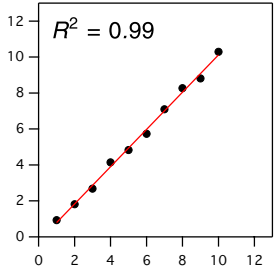
$$R^2 = 1 - \frac{\chi^2}{SS_{\text{tot}}}$$

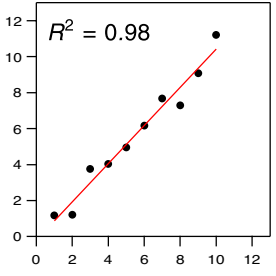
R^2 should lie between 0 and 1.

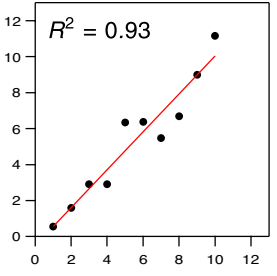
Interpreting R^2

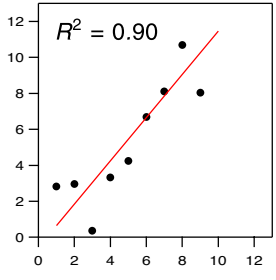
- $0 \leq R^2 \leq 1$
- $R^2 = 0$: No correlation between x and y in the experimental data.
- $R^2 = 1$: A “perfect” fit of the experimental data to the function.
- R^2 is the fraction of the total variation of the experimental y values that is accounted for by the linear function.

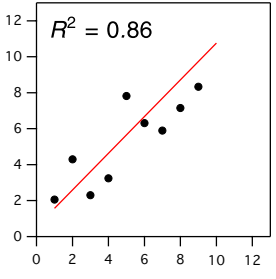
Some Examples

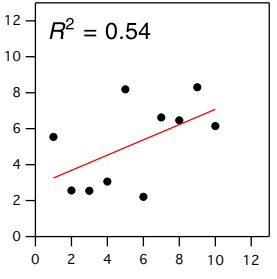












A Related Term: The Correlation Coefficient, r

- For a fit to the linear function, $y = mx + b$:

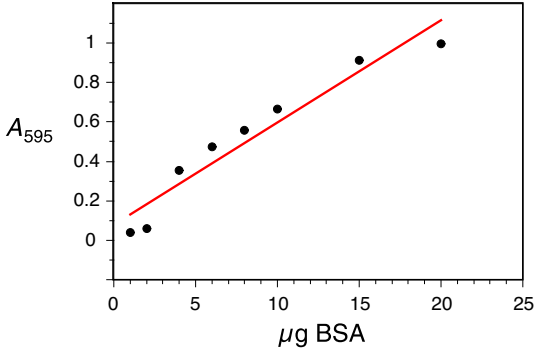
  $$R^2 = r^2$$

  where r is called the correlation coefficient, or Pearson correlation coefficient, or linear correlation coefficient.

- r can lie between -1 and 1.
  - If $r > 0$, y increases when x increases.
  - If $r < 0$, y decreases when x increases.
  - If $r = 0$, x and y are uncorrelated.
- r really only makes sense for the linear fit, but some programs will report it for other types of fits.
- R^2 can be calculated, and interpreted for a fit to any function:

Clicker Question #2

What if the fit isn't as good as we'd like?



$R^2 = 0.93$

Should we?

1. Delete some points?
2. Find a function that better represents the data?
3. Accept that there is some error in our measurements?
4. Repeat the experiment more carefully?

All answers count (for now)!

Polynomials as Fitting Functions

- General form of a polynomial function:

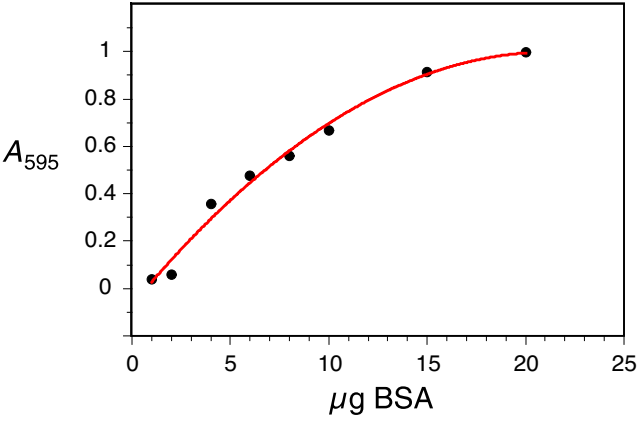
$$y = a_0 + a_1 x + a_2 x^2 + a_3 x^3 + \cdots + a_n x^n$$

- A polynomial in which the largest power of x is x^n is called an n^{th}-order polynomial.
  - A first-order polynomial is a straight line: $y = a_0 + a_1 x$
  - A second-order polynomial is also called a quadratic function:

    $y = a_0 + a_1 x + a_2 x^2$
  - A third-order polynomial is also called a cubic function:

    $y = a_0 + a_1 x + a_2 x^2 + a_3 x^3$
- An n^{th}-order polynomial contains $n + 1$ coefficients $(a_0, a_1, a_2, \ldots, a_n)$.
- A **minimum** of $n + 1$ data points are required to fit an n^{th}-order polynomial.

A 2nd-order Polynomial Least-squares Fit to Bradford Calibration Data



- For 2nd-order polynomial fit:

  $\chi^2 = 0.012$

  $R^2 = 0.988$

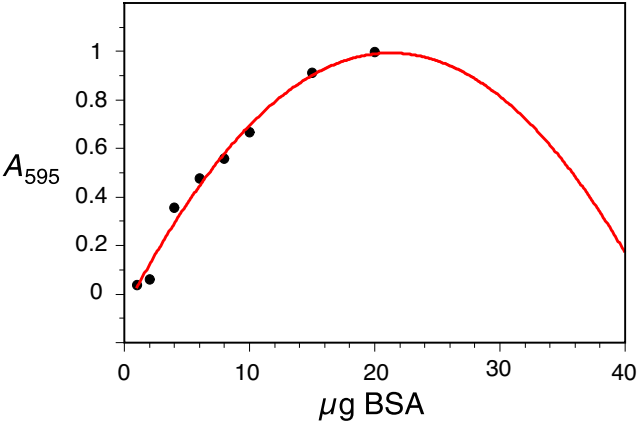
- For linear fit:

  $\chi^2 = 0.062$

  $R^2 = 0.93$

- Increasing the number of parameters almost always improves the fit!
- Is it justified here?

Does the Fit Function Make Sense Physically?



- Should the absorbance decrease as the amount of BSA increases beyond 20 μg?

  Probably not!

- The function serves as a calibration curve over the range used to fit it, but not beyond.