

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)

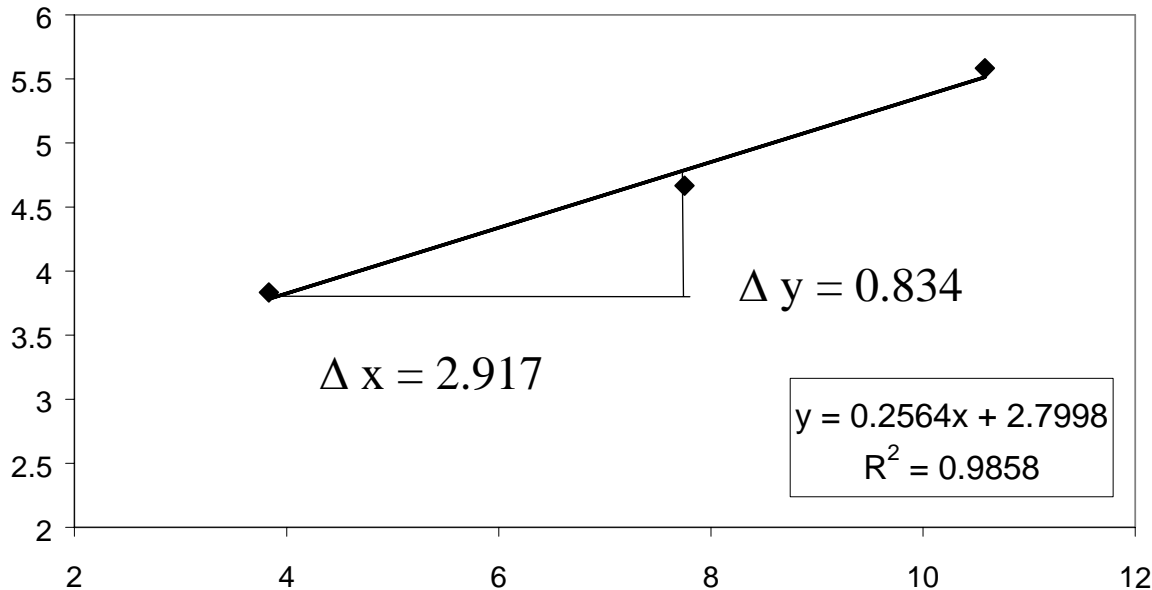
Least squares method web site:

<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 1st and 2nd points:

$$m = \Delta y / \Delta x$$
$$= 0.834 / 2.917$$
$$= 0.213$$

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!!!)

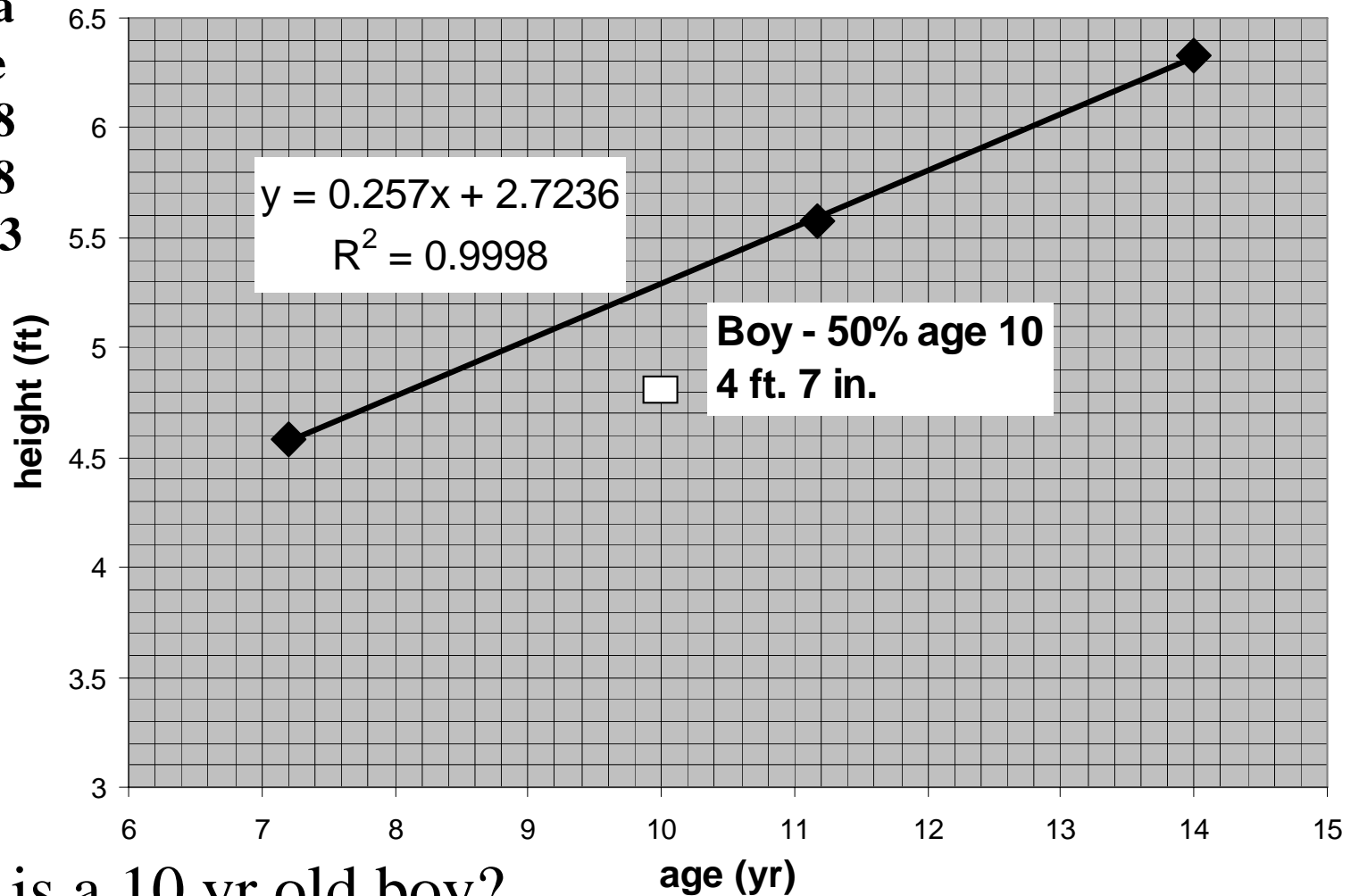
Correlation coefficient: Deviation of the each point from the calculated straight line, listed as an “r” value.

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 trendline (linear regression).

Calibr. Data

| age | size |
|------|------|
| 7.2 | 4.58 |
| 11.1 | 5.58 |
| 14.0 | 6.33 |



How tall is a 10 yr old boy?

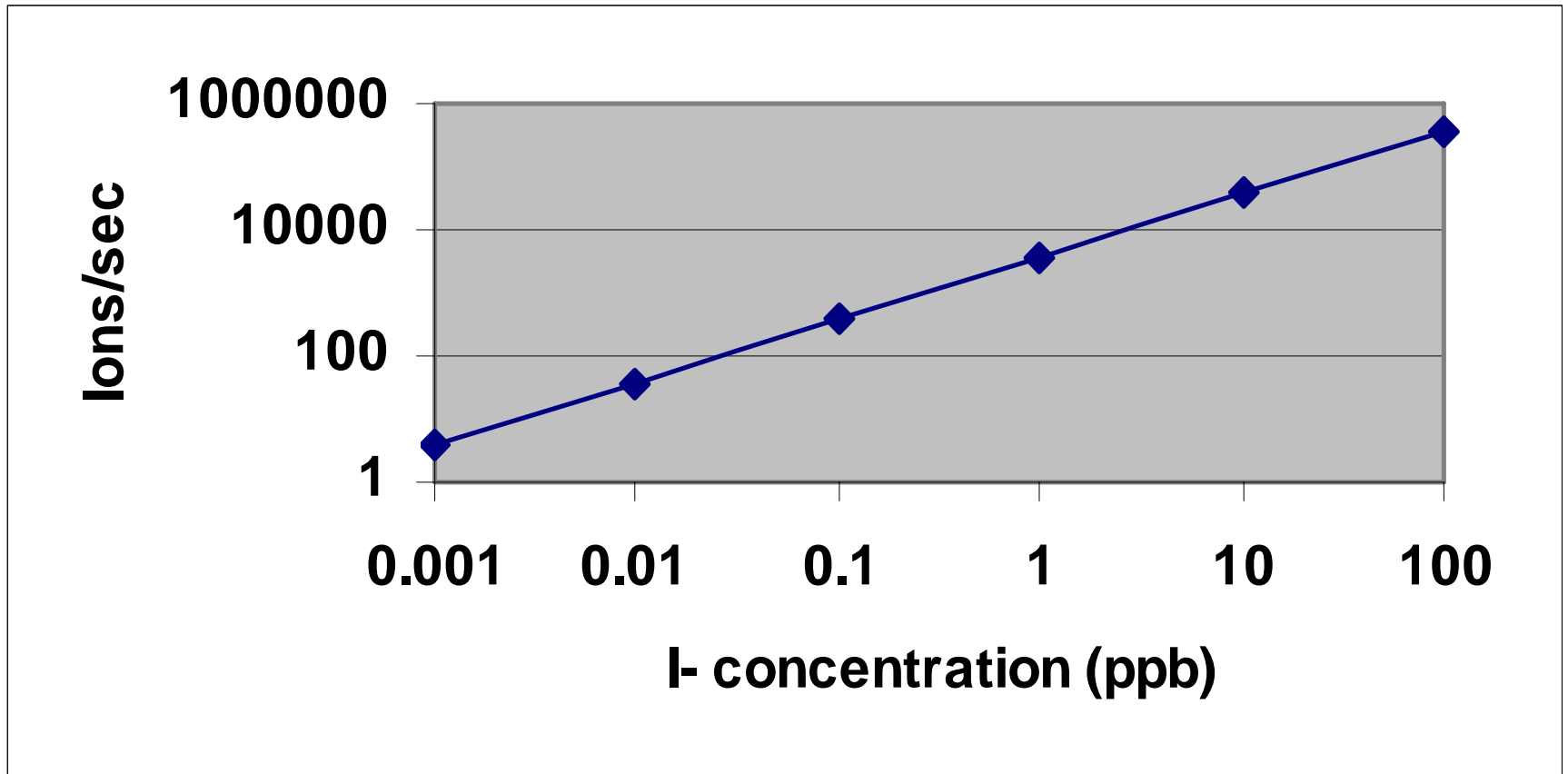
From growth charts at 50%, 10 yr old boy is 4.6 ft tall.

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

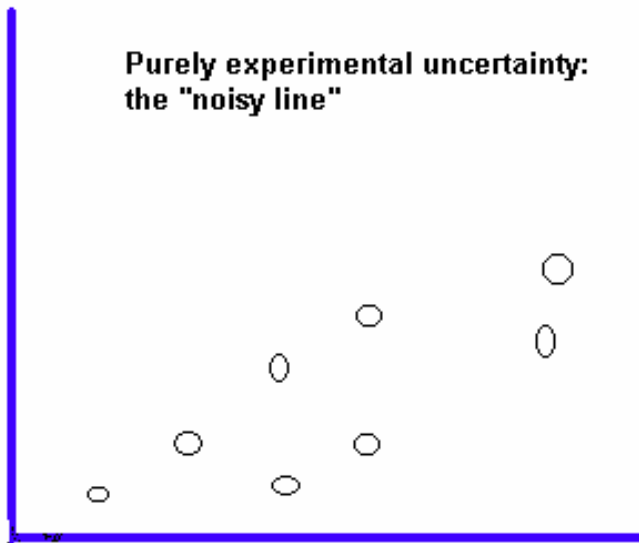


A few things to remember on linear regression

- One assumes errors in \mathbf{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 , r^2 should be examined

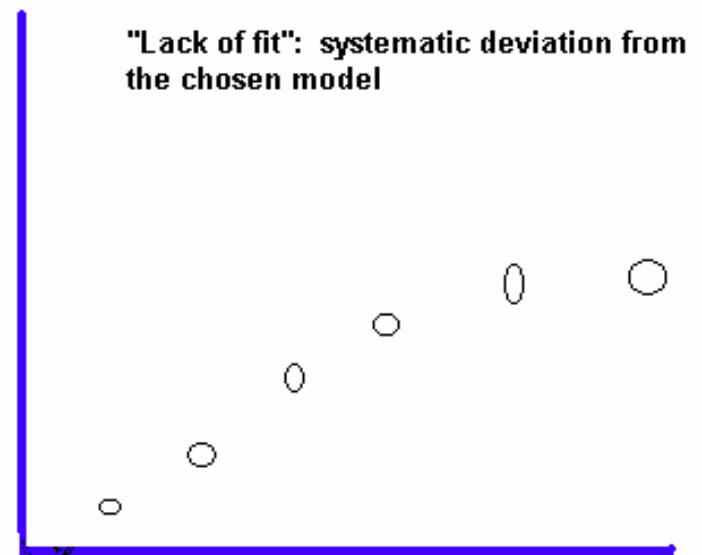
Two reasons why the line is bad: lack of fit, and purely experimental uncertainty

Analyte Signal / Internal Std Signal



Analyte Conc.

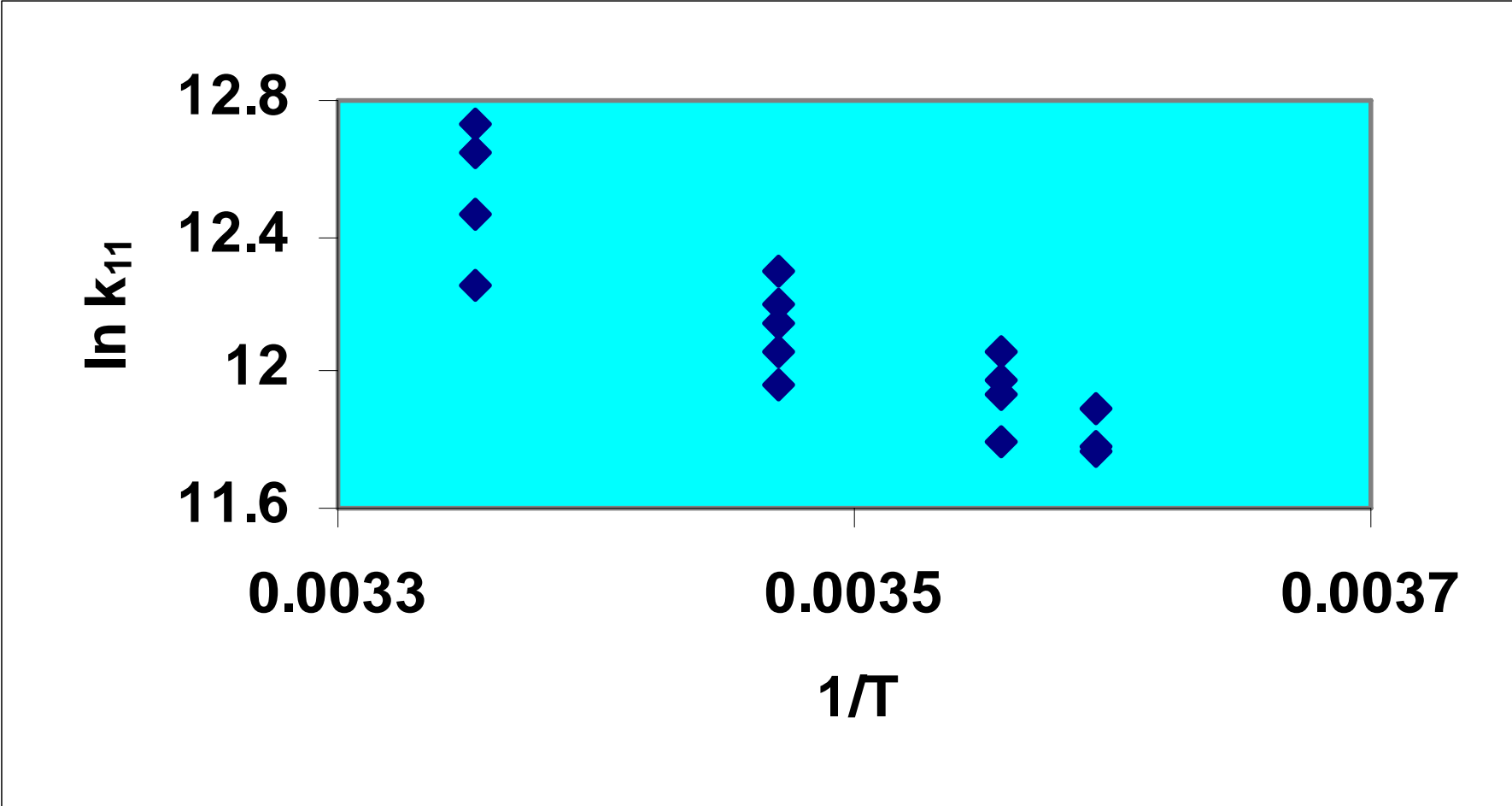
Analyte Signal / Internal Std Signal



Analyte Conc.

How to design the calibration set

- # settings for \mathbf{X} $>$ # parameters in model will allow “lack of fit” to be examined
(For example, linear fit: need at least 3 points)
- Replicate measurements of \mathbf{Y} for a given setting of \mathbf{X} will allow “purely experimental uncertainty” to be examined



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: $\text{Signal} = k * C_{\text{added}} + b$
- Equation is solved for concentration where **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:

$$I_{S+X} = k([S_f] + [X]_f)$$

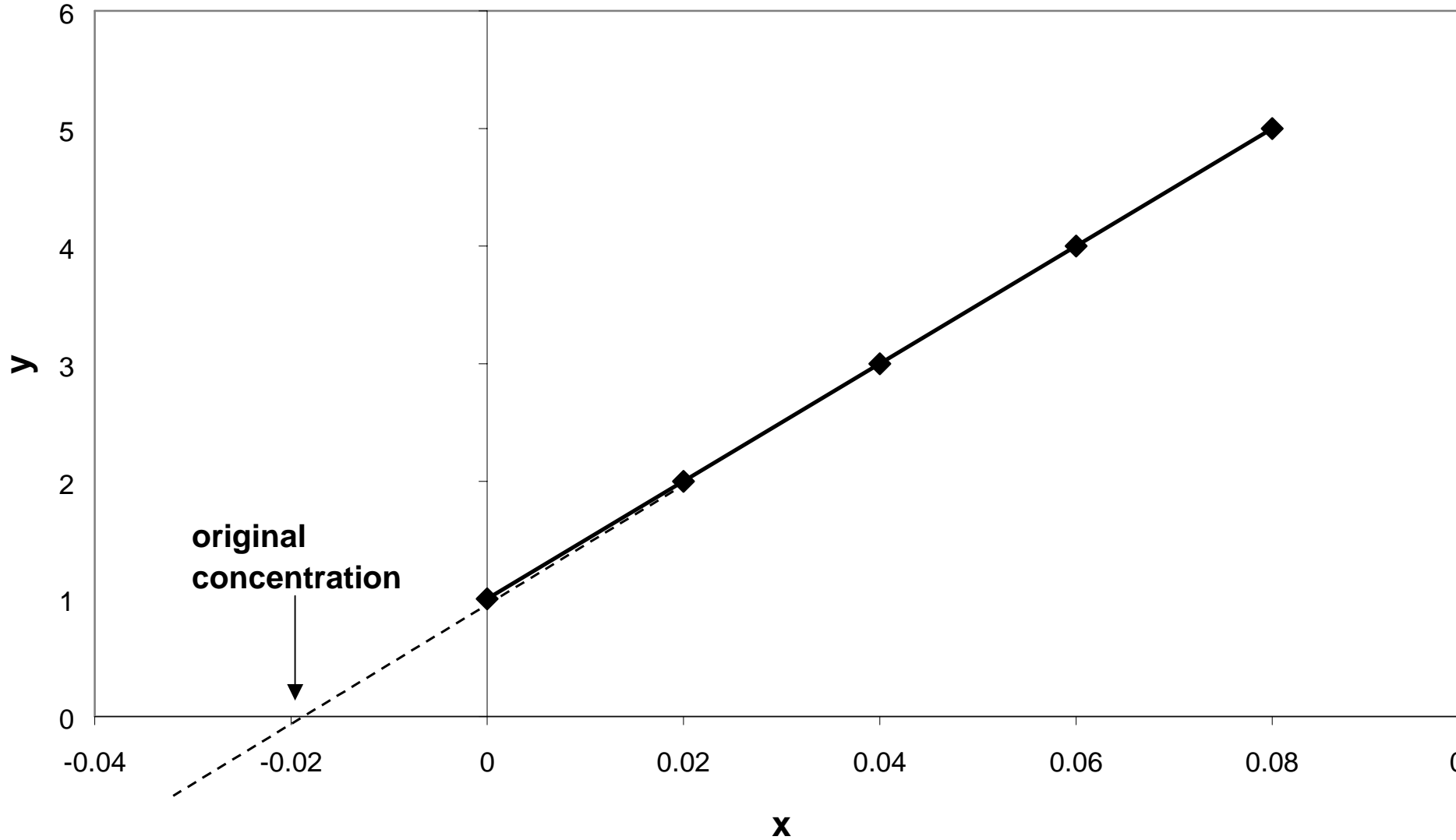
The signal intensity is proportional to the concentration of the analyte and the standard

$$\text{SO.... } I_x / I_{S+X} = k ([X_i] / k([S_f] + [X]_f)) = [X_i] / k([S_f] + [X]_f)$$

To get X_f and S_f : **MUST KNOW DILUTION FACTORS.**

$$[X_f] = [X_i] (V_i / V_f) \quad \text{and} \quad [S_f] = [S_i] (V_i / V_f)$$

Example of Standard Addition Plot



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)

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

Requirements for an internal standard

- Technique must be multicomponent - must separately measure signals for analyte and internal standard
- No interferences: analyte \rightleftharpoons internal std or sample matrix \rightleftharpoons 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.