INTRODUCTION TO INSTRUMENTAL ANALYSIS

OUTLINE:

Classification of Analytical Methods

Types of Instrumental Methods

Instruments for Analysis

Selecting an Analytical Method

Calibration of an Instrumental Methods

Definitions

Analytical Chemistry: The Science of Chemical Measurements.

***Analyte*:** The compound or chemical species to be measured, separated or studied.

Qualitative instrumental analysis is that measured property that indicates *presence* of analyte in matrix

Quantitative instrumental analysis is that magnitude of measured property that is proportional to *concentration* of analyte in matrix

CLASSICAL AND INSTRUMENTAL METHODS

CLASSICAL:

Qualitative - identification by color, indicators, boiling points, odors

Quantitative - mass or volume (e.g. gravimetric, volumetric)

INSTRUMENTAL:

Qualitative- chromatography, electrophoresis and identification by measuring physical property (e.g. spectroscopy, electrode potential)

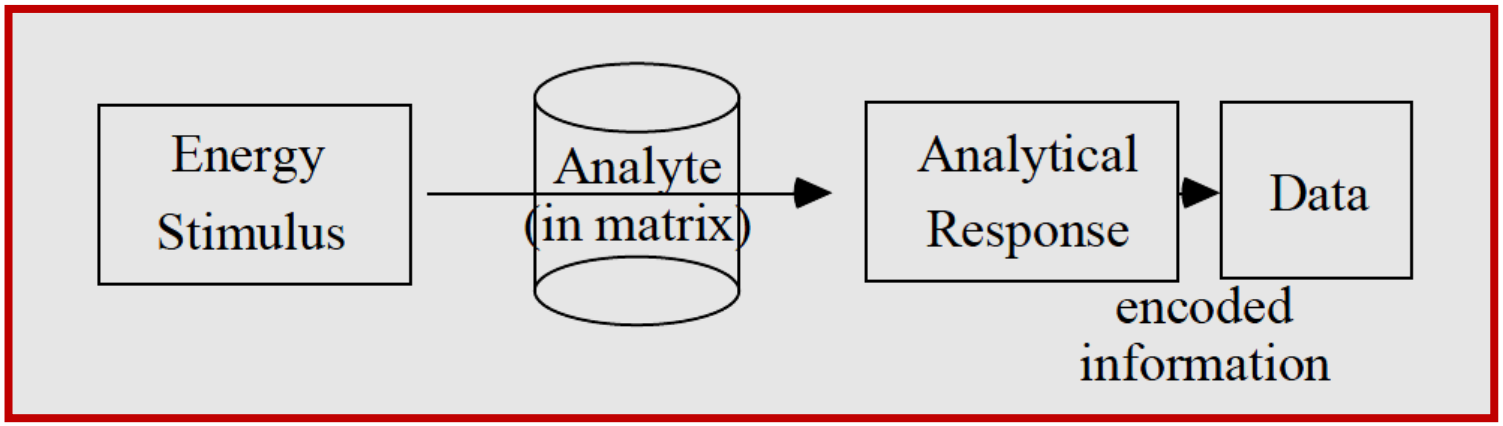
Quantitative- measuring property and determining relationship to concentration (e.g. spectrophotometry, mass spectrometry). Often, same instrumental method used for qualitative and quantitative analysis.

Types of Instrumental Methods

Example methods

Spectroscopy Technique	Radiation emission	Emission spectroscopy, fluorescence, phosphorescence, luminescence
	Radiation absorption	Absorption spectroscopy spectrophotometry, photometry, nuclear magnetic resonance NMR
Electrical Technique	Electrical potential	Potentiometry
	Electrical charge	Coulometry
	Electrical current	Voltammetry - amperometry, polarography
	Electrical resistance	Conductometry
Thermal Technique	Thermal	Thermal gravimetry, calorimetry

Instrumental Methods

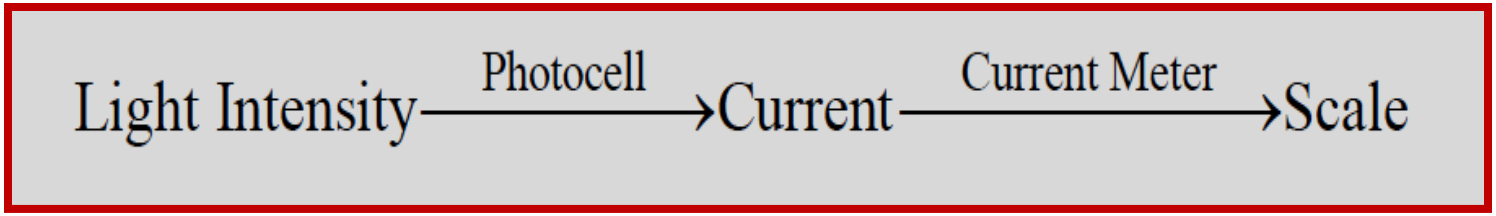


Block diagram for the overall process of instrumental measurement.

Example:	**spectrophotometry**
Instrument:	**spectrophotometer**
Stimulus:	**monochromatic light energy**
Analytical response:	**light absorption**
Transducer:	**photocell**
Data:	**electrical current**
Data processor:	**current meter**
Readout:	**meter scale**

DATA DOMAINS: way of encoding analytical response in electrical or non-electrical signals.

Interdomain conversions: transform information from one domain to another.



Detector
device that indicates change in environment

Transducer
device that converts non-electrical to electrical data

Sensor
device that detects physical, chemical or biological properties to electrical data

Block diagram of a fluorometer

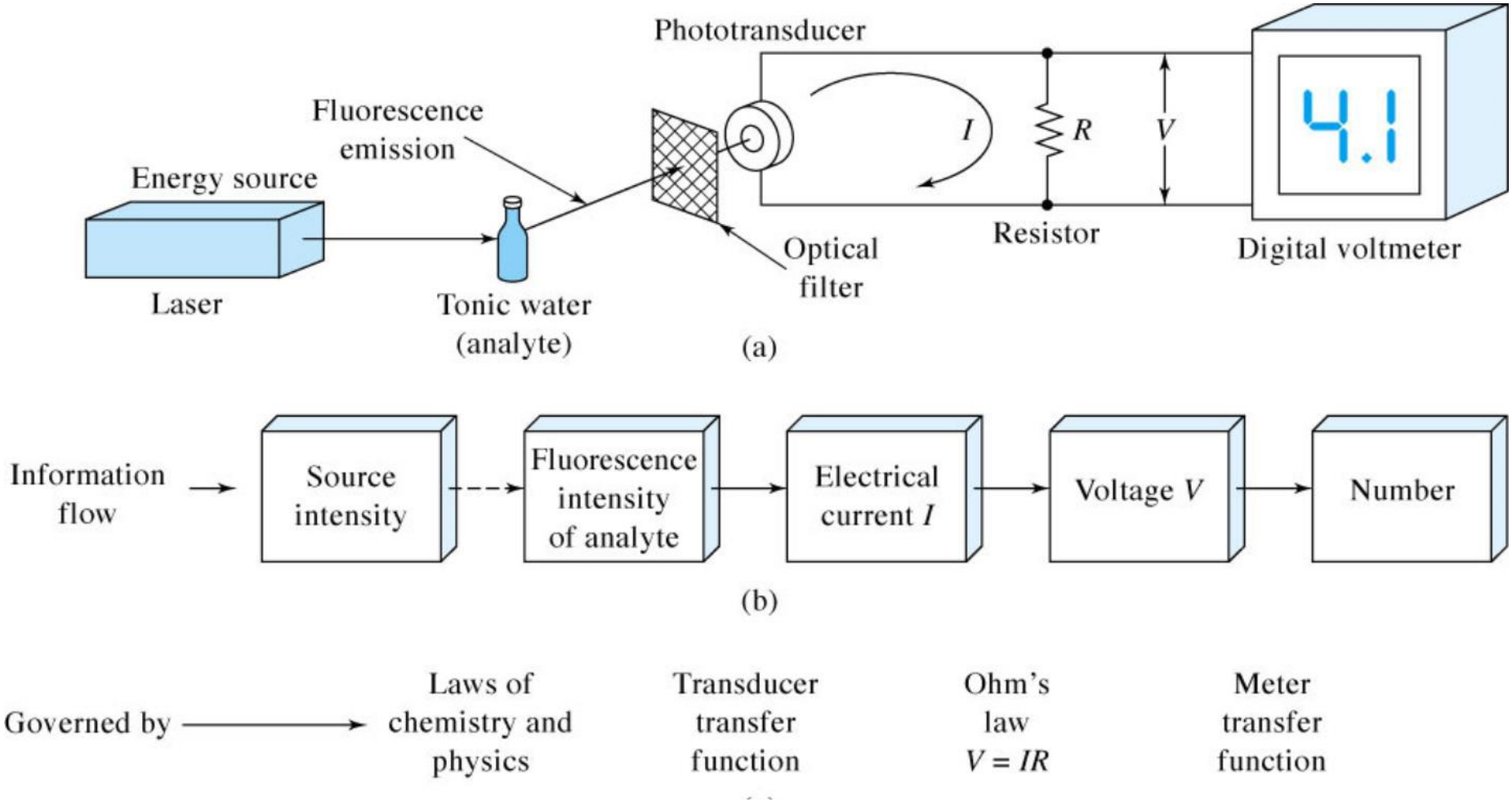


FIGURE 1-3 A block diagram of a fluorometer showing (a) a general diagram of the instrument, (b) a diagrammatic representation of the flow of information through various data domains in the instrument, and (c) the rules governing the data domain transformations during the measurement process.

Figures of Merit

Performance Characteristics: Figures of Merit

How to choose an analytical method? How good is measurement?

How well a calibration curve follows a straight line?- **Linearity**.

How reproducible? – **Precision**

How close to true value? - **Accuracy/Bias**

How small a difference can be measured? – **Sensitivity**

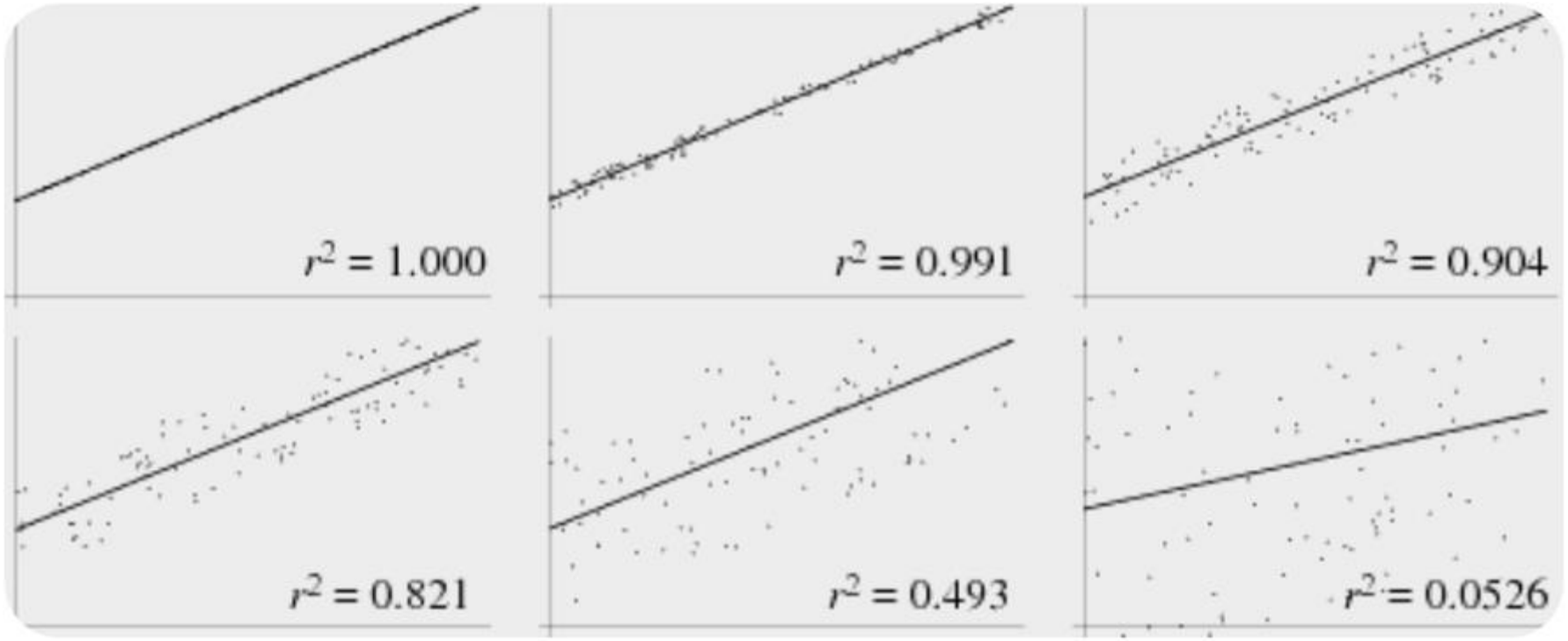
What range of amounts? - **Dynamic Range**

How much interference? - **Selectivity**

FIGURES OF MERIT: Linearity

- How well a calibration curve follows a straight line.
- R^2 (Square of the correlation coefficient)

$$R^2 = \frac{[\sum(x_i - \bar{x})(y_i - \bar{y})]^2}{\sum(x_i - \bar{x})^2 \sum(y_i - \bar{y})^2}$$


$r^2 = 1.000$
$r^2 = 0.991$
$r^2 = 0.904$
$r^2 = 0.821$
$r^2 = 0.493$
$r^2 = 0.0526$

FIGURES OF MERIT: PRECISION

INDETERMINATE OR RANDOM ERRORS

Standard deviation: $$s = \sqrt{\frac{\sum_{i-0}^{i=N} (x_i - \bar{x})^2}{N-1}}$$

Variance: s^2

Relative standard deviation: $$RSD = \frac{s}{\bar{x}}$$

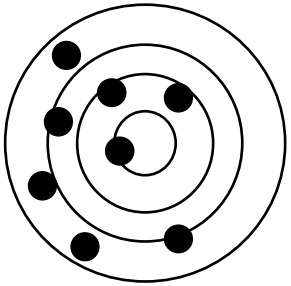
Standard deviation of mean: $$s_m = \frac{s}{\sqrt{N}}$$

FIGURES OF MERIT: ACCURACY

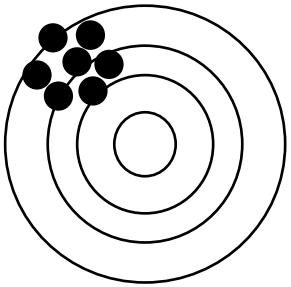
DETERMINATE ERRORS (operator, method, instrumental)

Bias: $\text{bias} = \bar{x} - x_{\text{true}}$

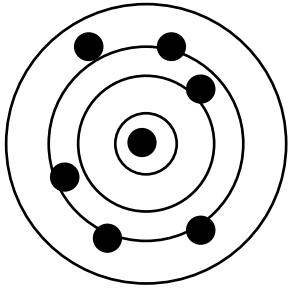
Illustrating the difference between "accuracy" and "precision"



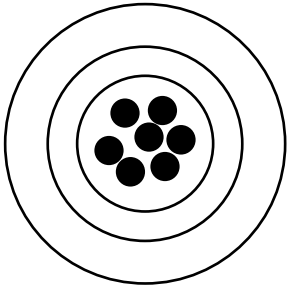
Low accuracy, low precision



Low accuracy, high precision



High accuracy, low precision



High accuracy, high precision

FIGURES OF MERIT: SENSITIVITY

Indicates the response of the instrument to changes in analyte concentration or a measure of a method's ability to distinguish between small differences in concentration in different samples. Effected by slope of calibration curve.

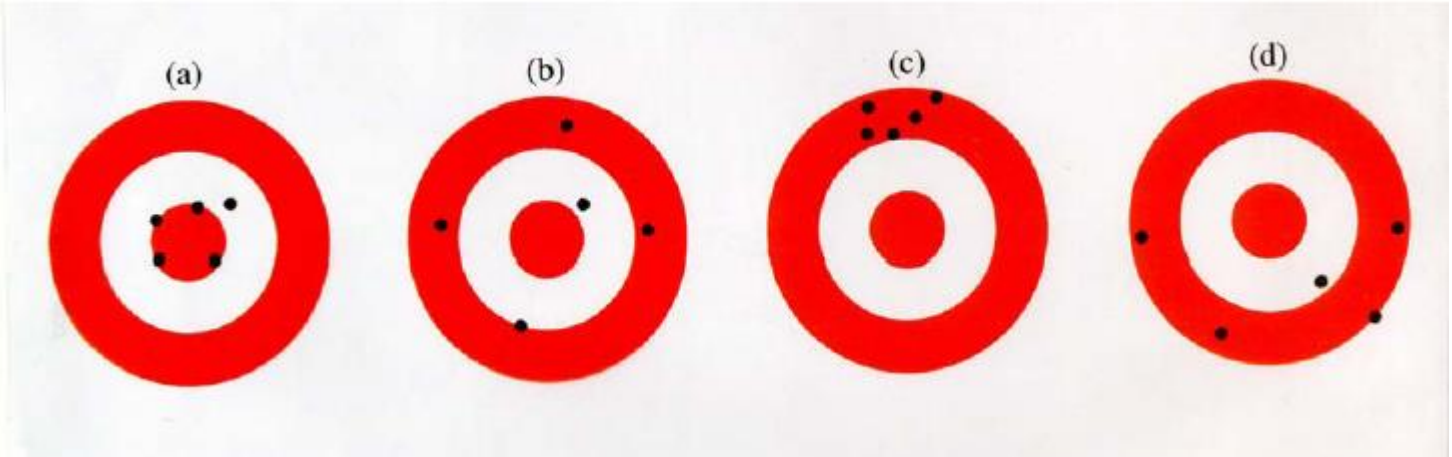
Sensitivity = γ= m/Ss

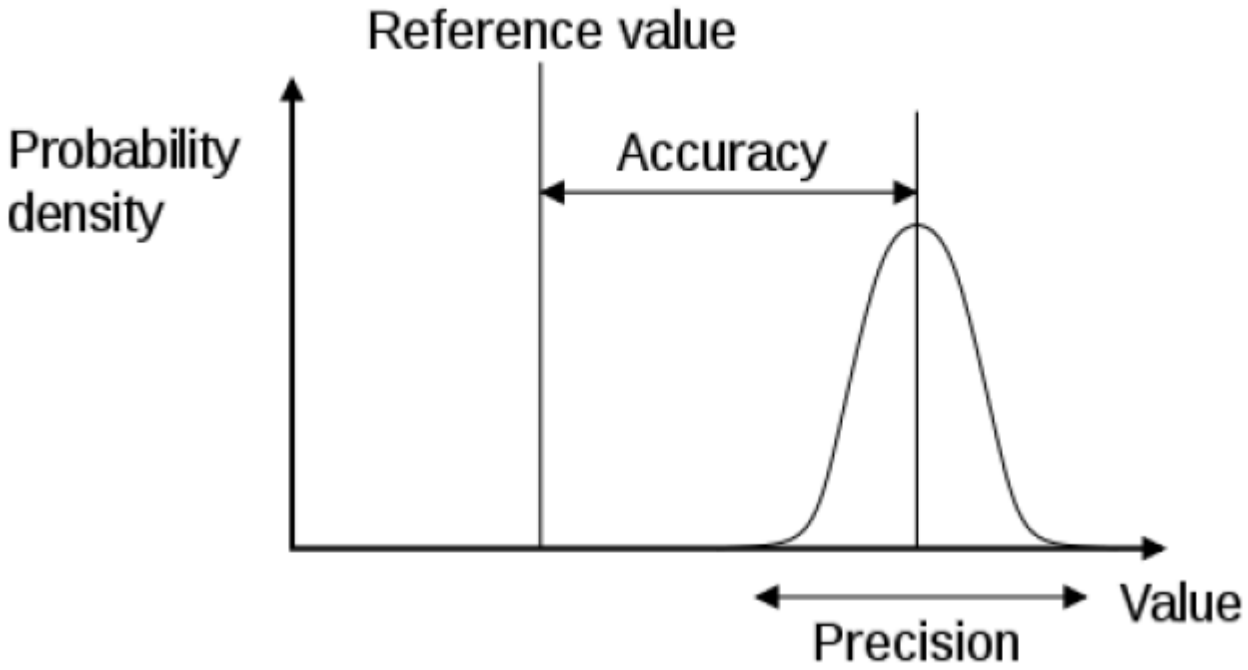
m = slope;

Ss is the standard deviation of measurement

(larger slope of calibration curve m, more sensitive measurement)

Accuracy vs Precision





FIGURES OF MERIT: LIMIT of DETECTION (LOD)

Signal must be bigger than random noise of blank

Minimum signal: $\text{Signal}_{min} = \text{Av. Signal}_{blank} + ks_{blank}$

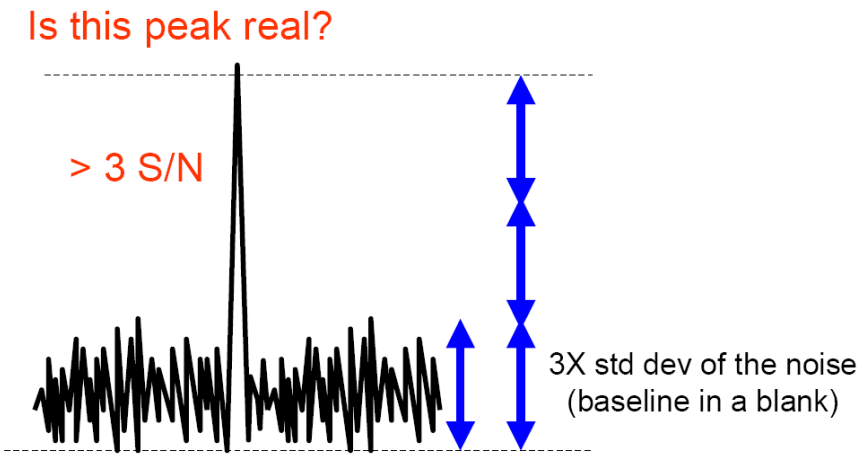
From statistics k=3 or more (at 95% confidence level)

Typically 3 times the signal-to-noise (based on standard deviation of the noise)

or

LOD =3 x Standard deviation of blank/ Slope

LOD=3 xS_b/m



FIGURES OF MERIT: DYNAMIC RANGE

At limit of detection we can say confidently analyte is present but cannot perform reliable quantitation

LOQ (limit of quantification): [lowest] at which quantitative measurements can reliably be made. Equal to 10 x Average Signal for blank *i.e.* 10Sbl. or

LOQ =10 x Standard deviation of blank/ Slope

LOQ=10 xSb/m

Limit of linearity (LOL): when signal is no longer proportional to concentration

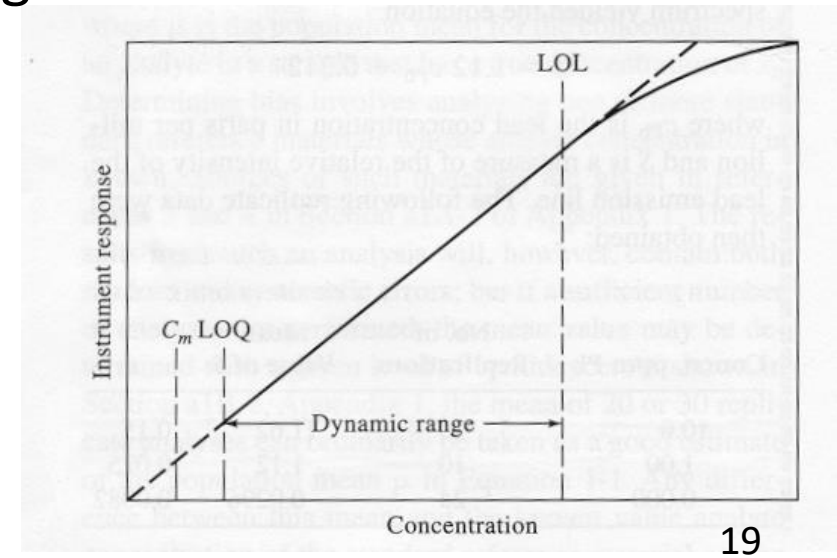
Dynamic range: the maximum range over which an accurate measurement can be made

From limit of quantitation to limit of linearity

LOQ: 10 s of blank

LOL: 5% deviation from linear

$$\text{Dynamic range: } \frac{\text{LOL}}{\text{LOQ}} \qquad 10^2 \text{ to} > 10^6$$



Example:

An analysis of the calibration data for the determination of lead based upon its flame emission spectrum yielded an equation: $S = 1.12C + 0.312$ where C is the Pb concn in ppm and S is a measure of the relative emission intensity. The following replicate data were obtained:

Concn (C), ppm	No. of replicates	Mean value of S	Std. dev.
10.0	10	11.62	0.15
1.00	10	1.12	0.025
0.000	24	0.0296	0.0082

Calculate (a) the calibration sensitivity, (b) the analytical sensitivity at 1 and 10 ppm of Pb,(c) the limit of detection limit (LOD), and ,(d) the limit of quantification limit (LOQ)

Example Solution:

a) calibration sensitivity is the slope of the calibration curve = 1.12

b) Using $\gamma = m/S_s$: at 10 ppm $\gamma = 1.12 / 0.15 = 7.5$ at 1 ppm

$\gamma = 1.12 / 0.025 = 45$

c) LOD= 3 x Sb/ slope= 3 x 0.0082/1.12 = 0.0219

d) LOQ= 10 x Sb/ slope= 10 x 0.0082/1.12 = 0.0732

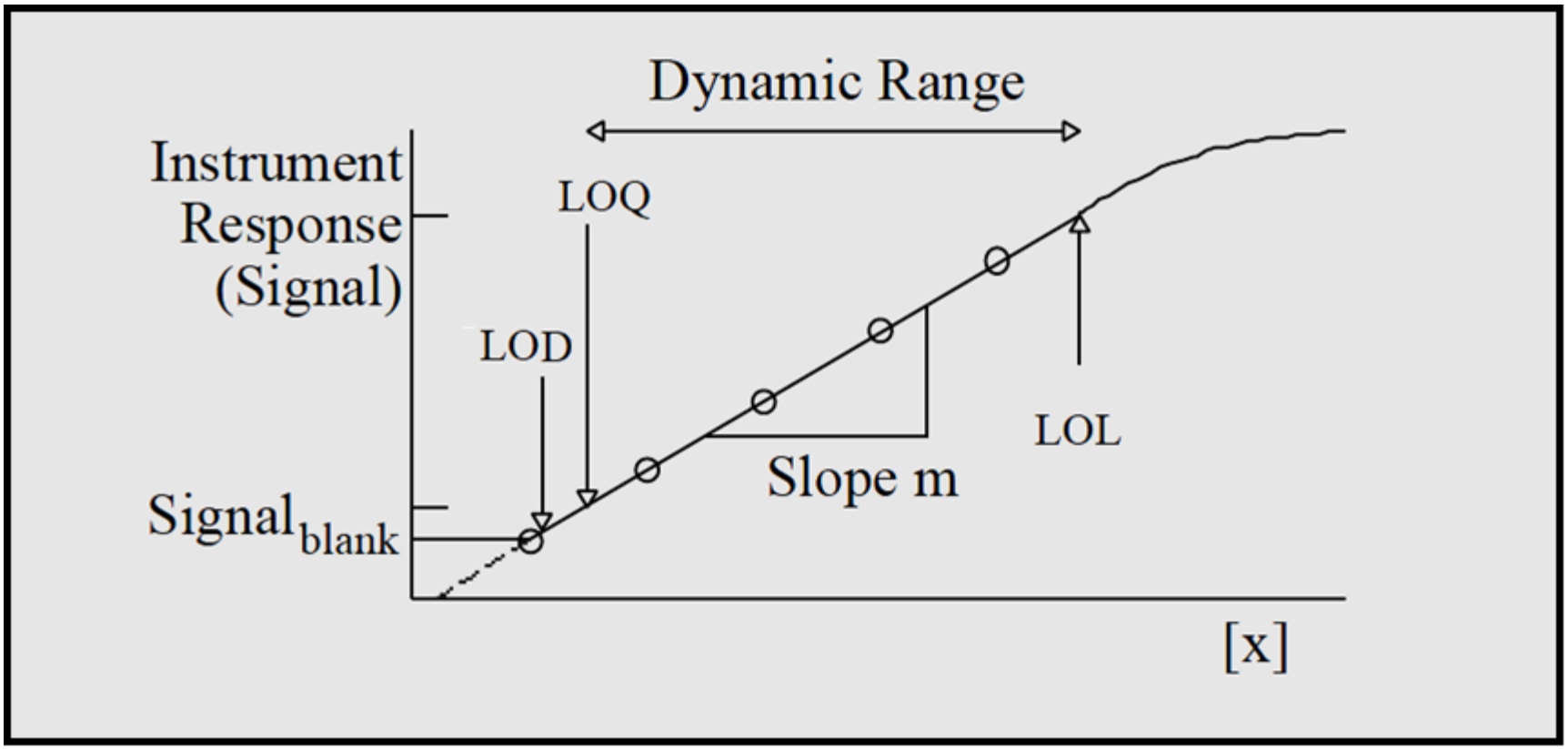
CALIBRATION METHODS

Basis of *quantitative* analysis is magnitude of *measured property* is proportional to *concentration* of analyte

$$\text{Signal} \propto [x] \quad \text{or} \quad \text{Signal} = m[x] + \text{Signal}_{\text{blank}}$$

$$[x] = \frac{\text{Signal} - \text{Signal}_{\text{blank}}}{m}$$

CALIBRATION CURVES (WORKING or ANALYTICAL)



SAMPLE PROBLEM:

Analyte Concentration (ppm*)	Absorbance
0.0 (blank)	0.05
0.9	0.15
2.0	0.24
3.1	0.33
4.1	0.42

Define Variance and Covariance:

$$S_{xx} = \frac{\sum (x_i - \bar{x})^2}{N-1} \qquad S_{xy} = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{N-1}$$

$$\bar{x} = 2.02 \qquad \bar{y} = 0.238$$

$$S_{xx} = \frac{\left(2.02^2 + 1.12^2 + 0.02^2 + 1.08^2 + 2.08^2\right)}{4} = \frac{10.828}{4} = 2.707$$

$$S_{xy} = \frac{(-2.02 \times -0.188) + (-1.12 \times -0.088) + (-0.02 \times 0.002) + \ldots}{4}$$

$$= \frac{0.9562}{4} = 0.23905$$

Slope: $m = \frac{S_{xy}}{Sx} = \frac{0.23905}{2.707} = 0.0883$

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

Intercept: $= 0.238 - (0.0883 \times 2.02)$

$= 0.0596$

Calibration expression is

Absorbance=0.0883[Analyte (ppm)]+0.0596

Calibration Techniques

Calibration Techniques

- 1. External Calibration Curve Method
- 2. Standard Additions Method
- 3. Internal Standard Method

External Calibration Curve Method

1. Most convenient when a large number of similar samples are to be analyzed.

2. Most common technique.

3. Facilitates calculation of Figures of Merit.

External Calibration Curve Procedure

1. Prepare a series of standard solutions (analyte solutions with known concentrations).

2. Plot [analyte] vs. Analytical Signal.

3. Use signal for unknown to find [analyte].

Example: Pb in Blood by GFAAS

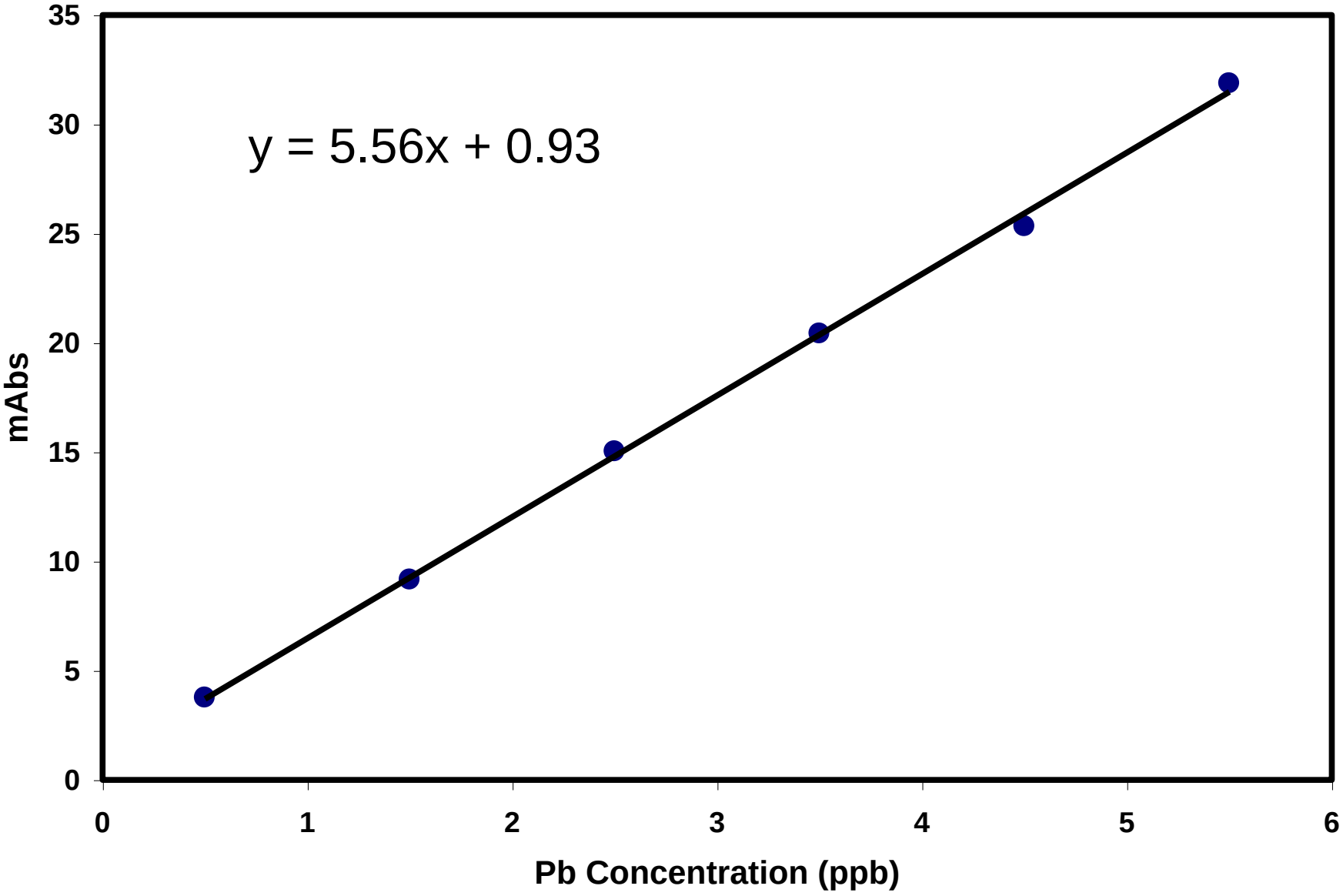
[Pb] (ppb)	Signal (mAbs)
0.50	3.76
1.50	9.16
2.50	15.03
3.50	20.42
4.50	25.33
5.50	31.87

Results of linear regression:

$S = mC \pm b$

$m = 5.56$ mAbs/ppb

$b = 0.93$ mAbs


y = 5.56x + 0.93
mAbs
Pb Concentration (ppb)
0
5
10
15
20
25
30
35
0
1
2
3
4
5
6

Calculate the LOD for Pb

20 blank measurements gives an average signal

0.92 mAbs

with a standard deviation (Sb) of

$$\sigma_{bl} = 0.36 \text{ mAbs}$$

LOD = 3 Sb/m = 3 x 0.36 mAbs / 5.56 mAbs/ppb

LOD = 0.2 ppb

Find the LOL for Pb

Lower end = LOD = 0.2 ppb

(include this point on the calibration curve)

S_{LOD} = 5.56 x 0.2 + 0.93 = 2.0 mAbs

(0.2 ppb (X) , 2.0 mAbs (Y))

Find the LOL for Pb

Upper end = collect points beyond the linear region and estimate the 95% point.

Suppose a standard containing 18.5 ppb gives rise to s signal of (Y practical= 98.52 mAbs)

This is approximately 5% below the expected value of (Y theoretical=103.71 mAbs)

(18.50 ppb , 98.52 mAbs)

Standard Addition Method
Reduce matrix effect

1. Most convenient when a small number of samples are to be analyzed.

2. Useful when the analyte is present in a complicated matrix and no ideal blank is available.

Standard Addition Procedure

1. Add one or more increments of a standard solution to sample aliquots of the same size. Each mixture is then diluted to the same volume.

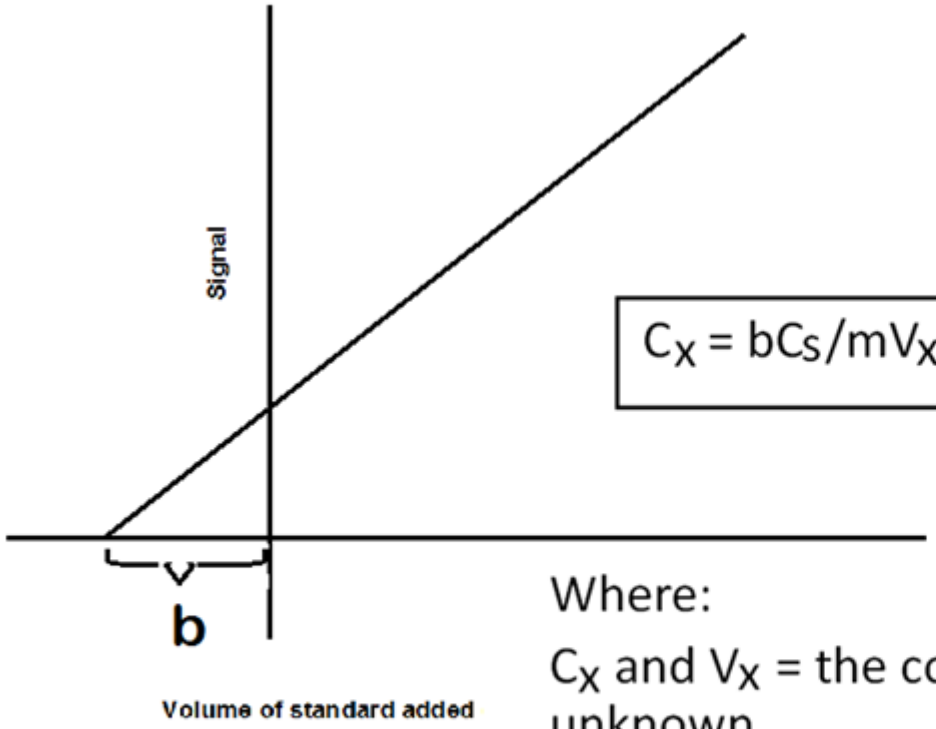
2. Prepare a plot of Analytical Signal versus:
   a) volume of standard solution added, or
   b) concentration of analyte added.

Standard Addition Procedure

3. The x-intercept of the standard addition plot corresponds to the amount of analyte that must have been present in the sample (after accounting for dilution).

4. The standard addition method assumes:
   a) the curve is linear over the concentration range
   b) the y-intercept of a calibration curve would be 0

Calculation of standard addition



$$C_X = bC_S/mV_X$$

Where:

C_X and V_X = the concentration and the volume of unknown

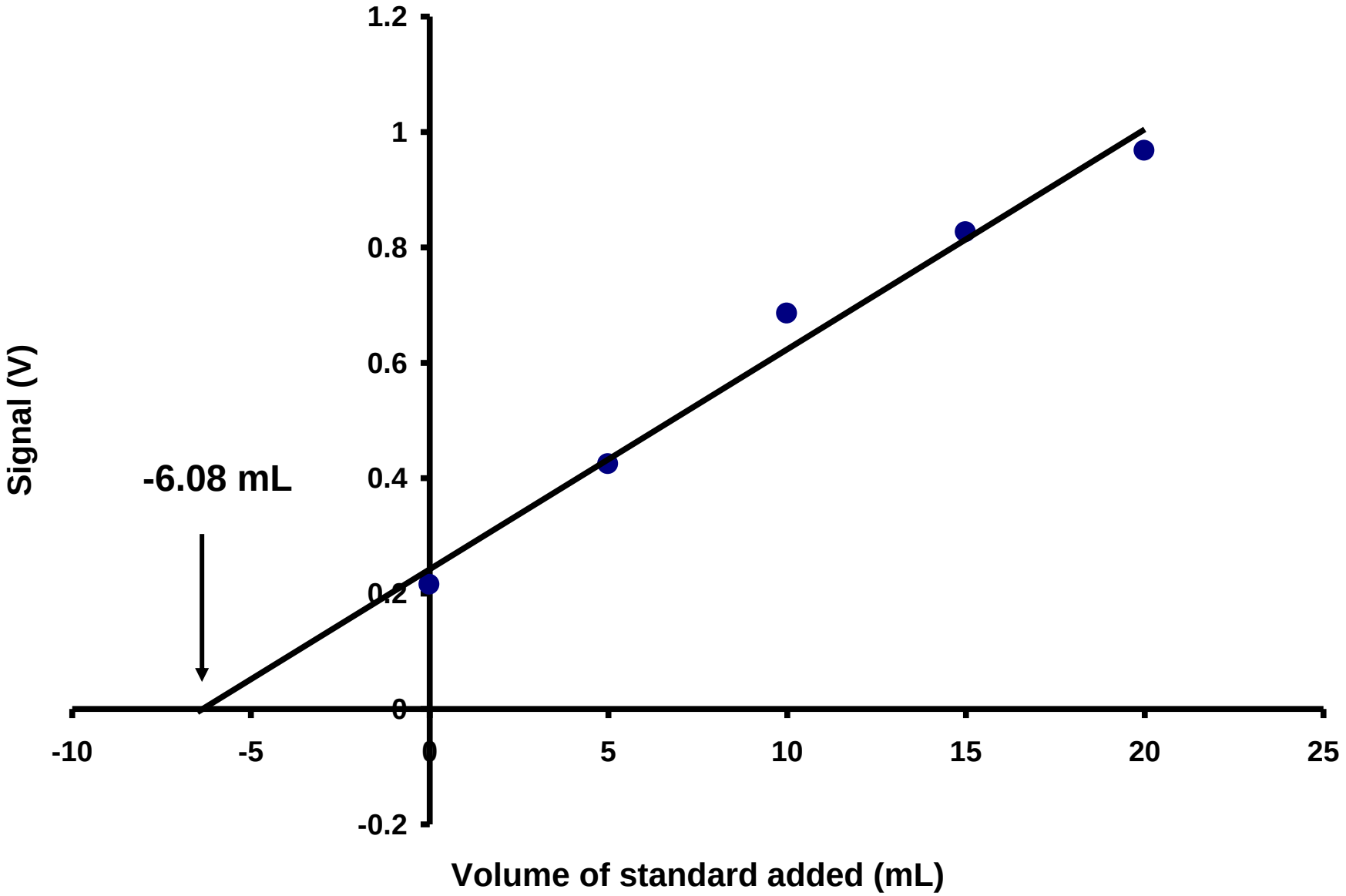
C_S and V_S = the concentration and the volume of added standard

Example: Fe in Drinking Water

Sample Volume (mL)	Standard Volume (mL)	Signal (V)
10	0	0.215
10	5	0.424
10	10	0.685
10	15	0.826
10	20	0.967

The concentration of the Fe standard solution is 11.1 ppm

All solutions are diluted to a final volume of 50 mL


1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-10
-5
0
5
10
15
20
25
Signal (V)
-6.08 mL
Volume of standard added (mL)

[Fe] = ?

x-intercept = -6.08 mL

Therefore, 10 mL of sample diluted to 50 mL would give a signal equivalent to 6.08 mL of standard diluted to 50 mL.

$$V_{sam} \times [Fe]_{sam} = V_{std} \times [Fe]_{std}$$

10.0 mL x [Fe] = 6.08 mL x 11.1 ppm

[Fe] = 6.75 ppm

Internal Standard Method
Reduce matrix and instrument effects

1. Most convenient when variations in analytical sample size, position, or matrix limit the precision of a technique.
2. May correct for certain types of noise.

Internal Standard Procedure

1. Prepare a set of standard solutions for analyte (A) as with the calibration curve method, but add a constant amount of a second species (B) to each solution.

2. Prepare a plot of S_A/S_B versus [A].

3. External calibration equation

$$S_A/S_B = mC + b$$

Notes

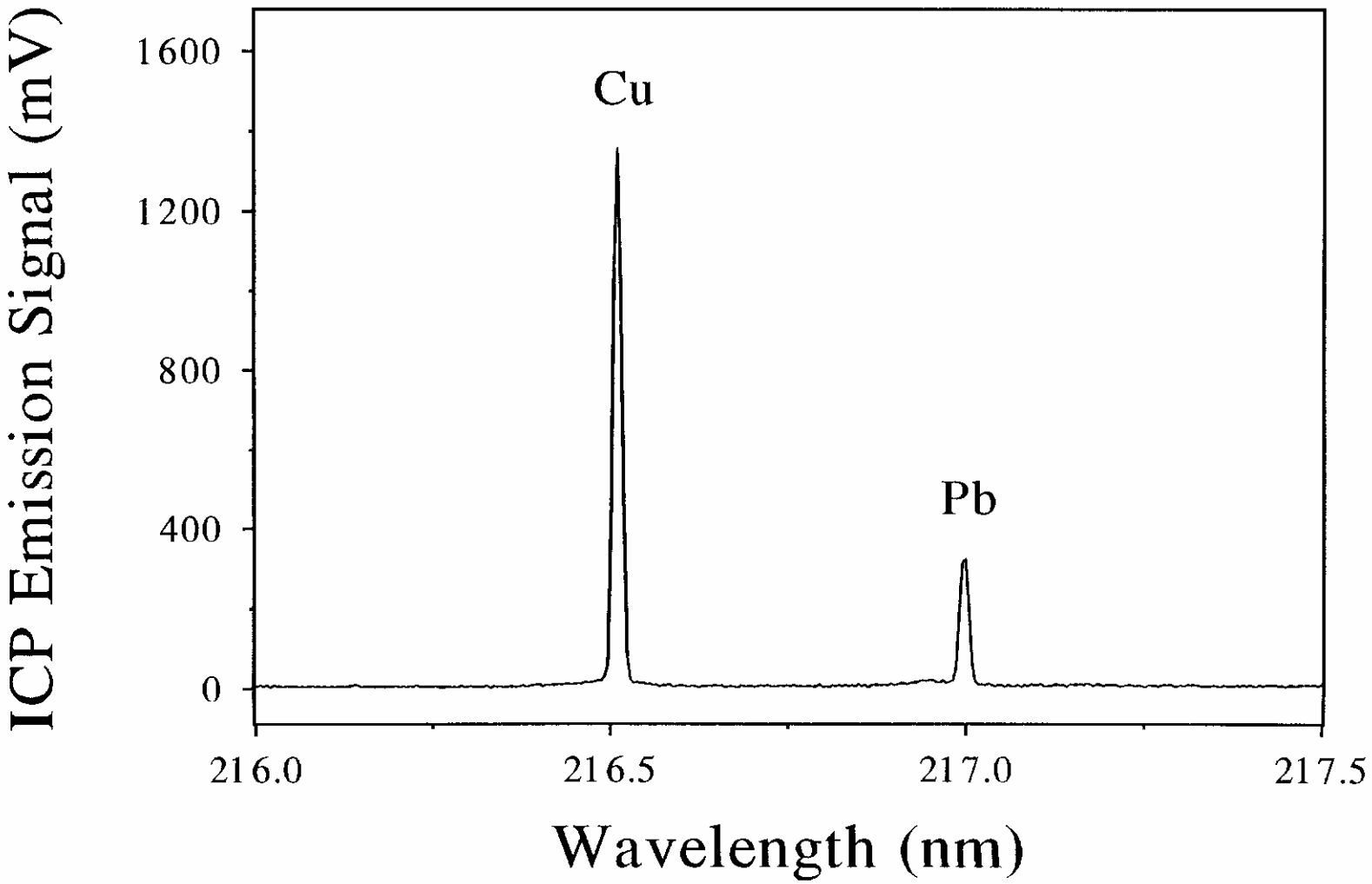
1. The resulting measurement will be independent of sample size and position.

2. Species A & B must not produce signals that interfere with each other. Usually they are separated by wavelength or time.

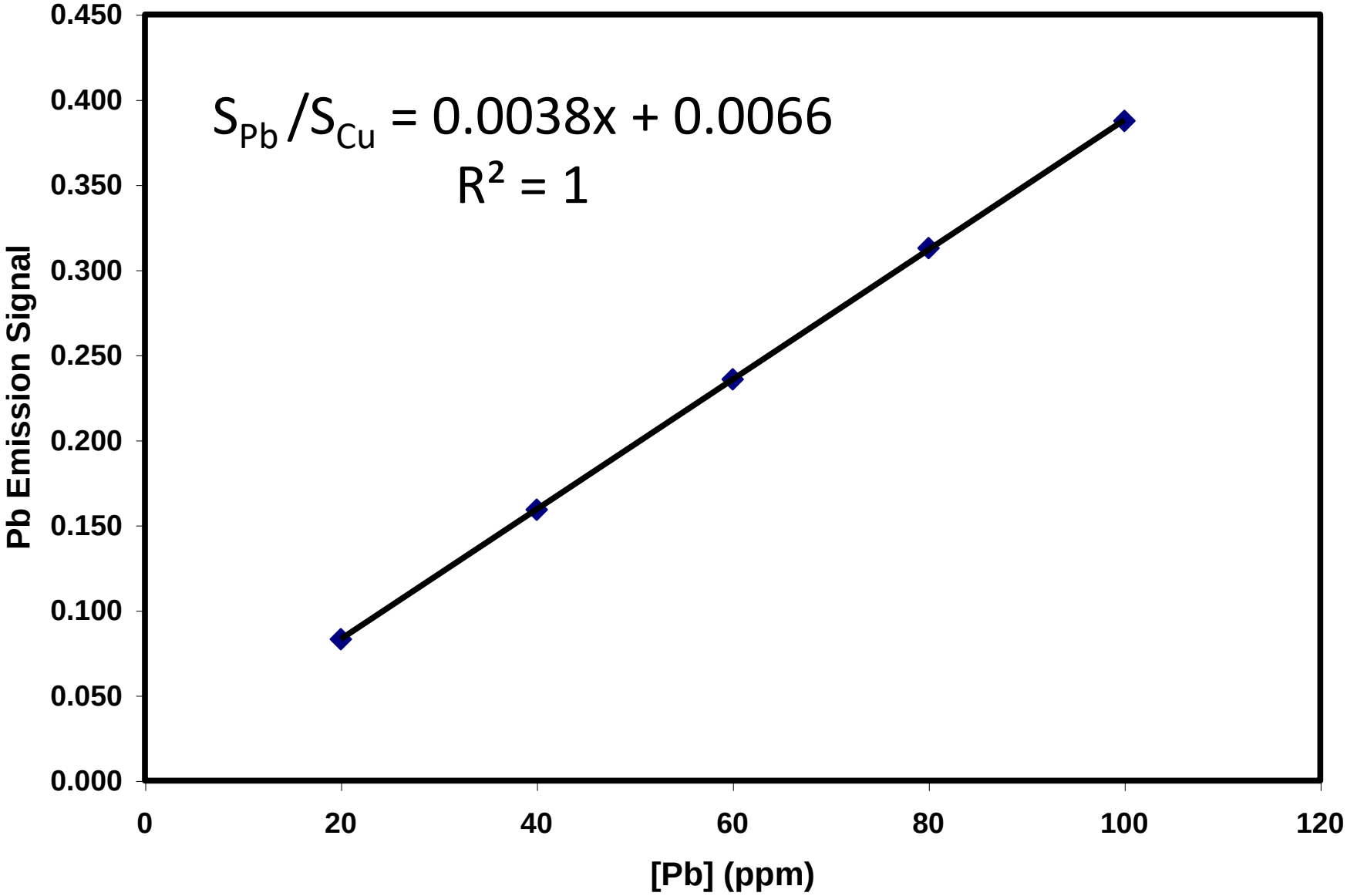
Example: Pb by ICP Emission

Each Pb solution contains
100 ppm Cu.

	Signal		
[Pb] (ppm)	Pb	100 ppm Cu	Pb/Cu
20	112	1347	0.083
40	243	1527	0.159
60	326	1383	0.236
80	355	1135	0.313
100	558	1440	0.388


ICP Emission Signal (mV)
1600
1200
800
400
0
Cu
Pb
216.0
216.5
217.0
217.5
Wavelength (nm)

Internal Standard Correction



Results for an unknown sample after adding 100 ppm Cu

	Signal		
Run	Pb	Cu	Pb/Cu
1	346	1426	0.243
2	297	1229	0.242
3	328	1366	0.240
4	331	1371	0.241
5	324	1356	0.239
mean	325	1350	0.241

$$0.241 = 0.0038\ X + 0.0066$$

$$X = 61.684 \text{ ppm of Pb}$$

Chapter 5
Signals and Noise

- **Signal** carries information about the analyte that is of interest to us.
- **Noise** is made up of extraneous information that is unwanted because it degrades the accuracy and precision of an analysis
- **Signal-to-Noise Ratio**

  S/N = (mean)/(Standard deviation) = $\frac{\bar{x}}{s} = \frac{1}{RSD}$

  Signal-to-noise (S/N) is much more useful figure of merit than noise alone for describing the quality of an analytical method. The magnitude of the noise is defined as the standard deviation s of numerous measurements and signal is given by the mean $\bar{x}$ of the measurements. S/N is the reciprocal of the relative standard deviation. S/N < 2 or 3 → impossible to detect a signal.

Sources of Noise

Analysis are affected by two types of noise:

1. Chemical noise
2. Instrumental noise

Chemical noise: Arises from an uncontrollable variables that effect the chemistry of the system being analyzed. Examples are undetected variations in temperature, pressure, chemical equilibria, humidity, light intensity etc.

Instrumental Noise: Noise is associated with each component of an instrument – i.e., with the source, the input transducer, signal processing elements and output transducer. Noise is a complex composite that usually cannot be fully characterized. Certain kinds of instrumental noise are recognizable, such as:

1. Thermal or Johnson noise
2. Shot noise
3. Flicker or 1/f noise
4. Environmental noise

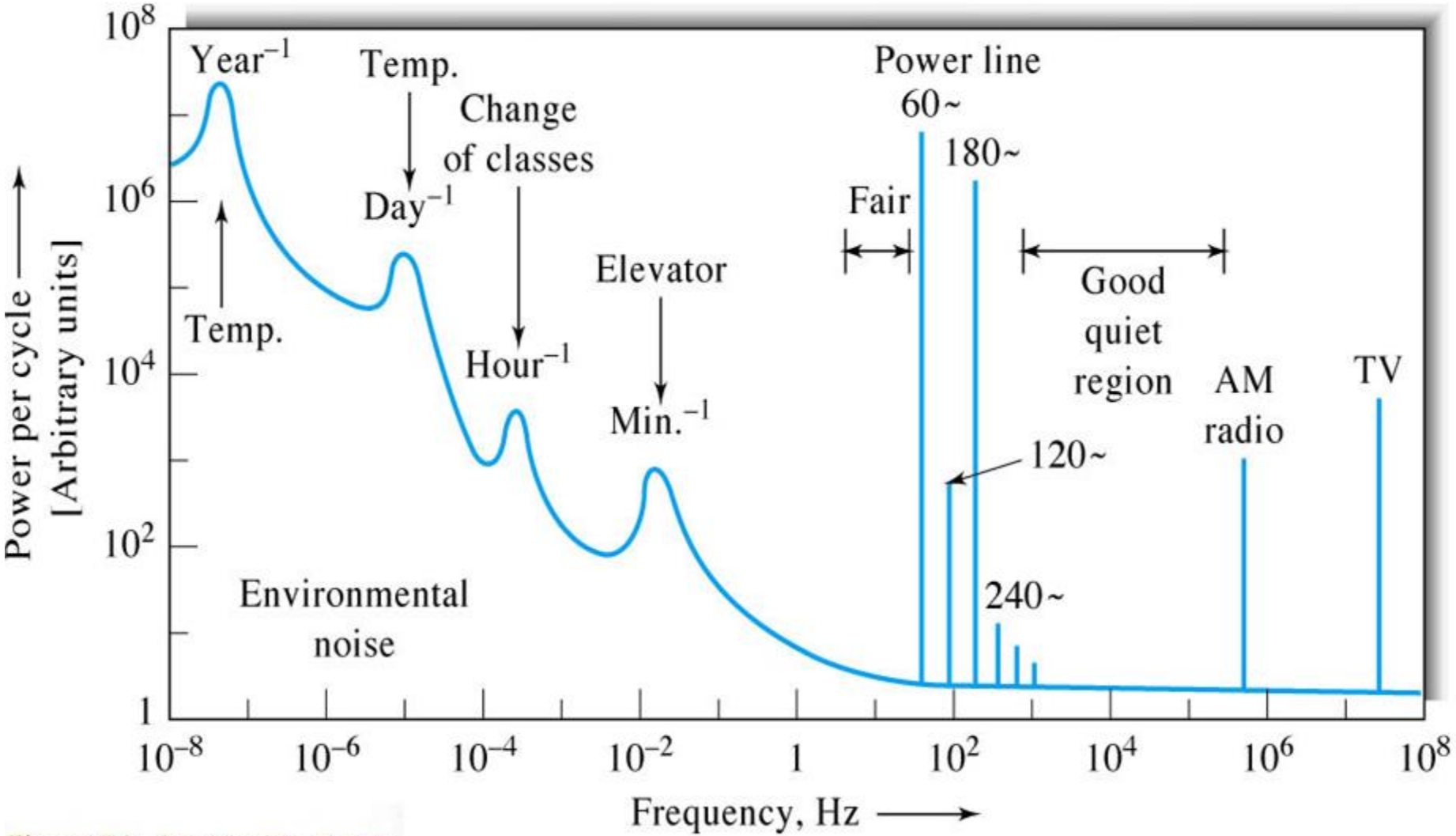


Figure 5-3 Some sources of environmental noise in a university laboratory. Note the frequency dependence and regions where various

Signal-to-Noise Enhancement: When the need for sensitivity and accuracy increased, the signal-to-noise ratio often becomes the limiting factor in the precision of a measurement. Both **hardware** and **software** methods are available for improving the signal-to-noise ratio of an instrumental method.

Software Methods

- Ensemble Average
  - Average of spectra
  - Average can also be sum of collected spectra
- Boxcar average
  - Average of points in a spectra

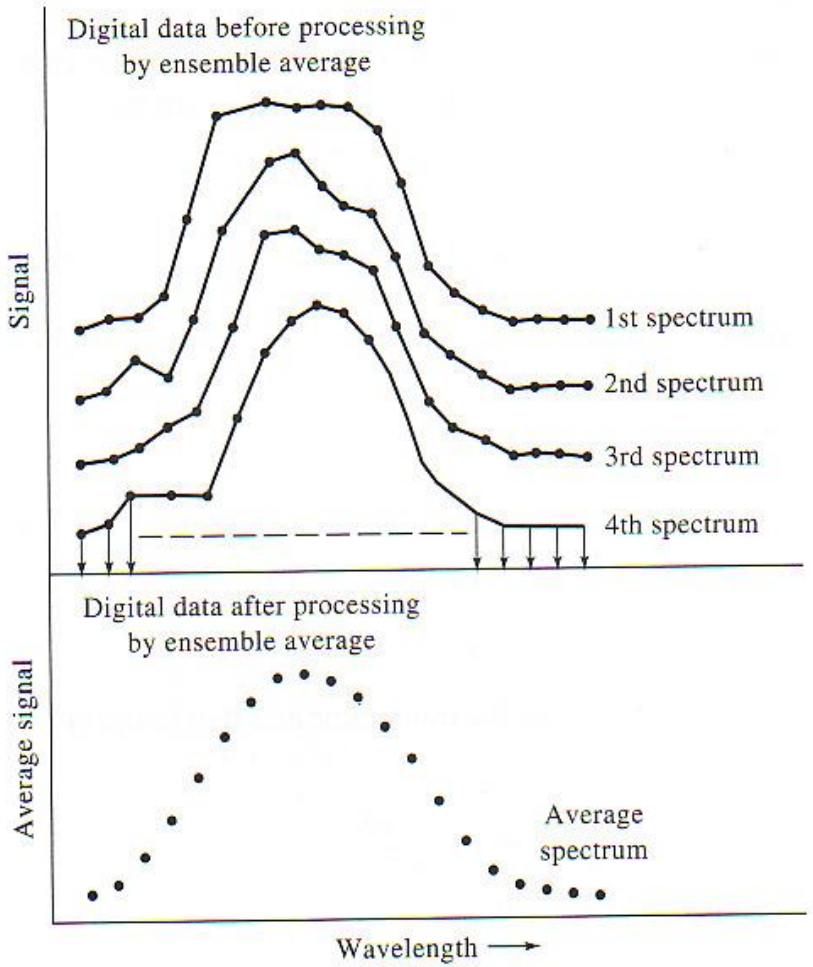


Figure 5-9 Ensemble averaging of a spectrum. *(From D. Binkley and R. Dessy,* J. Chem. Educ., *1979, 6, 150. With permission.)*

Software Methods

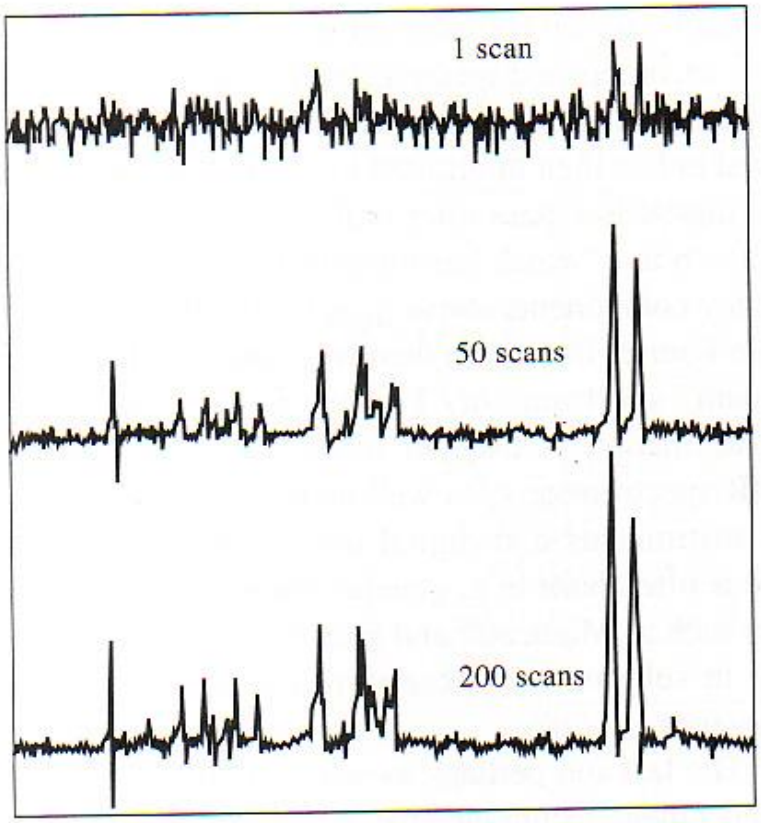
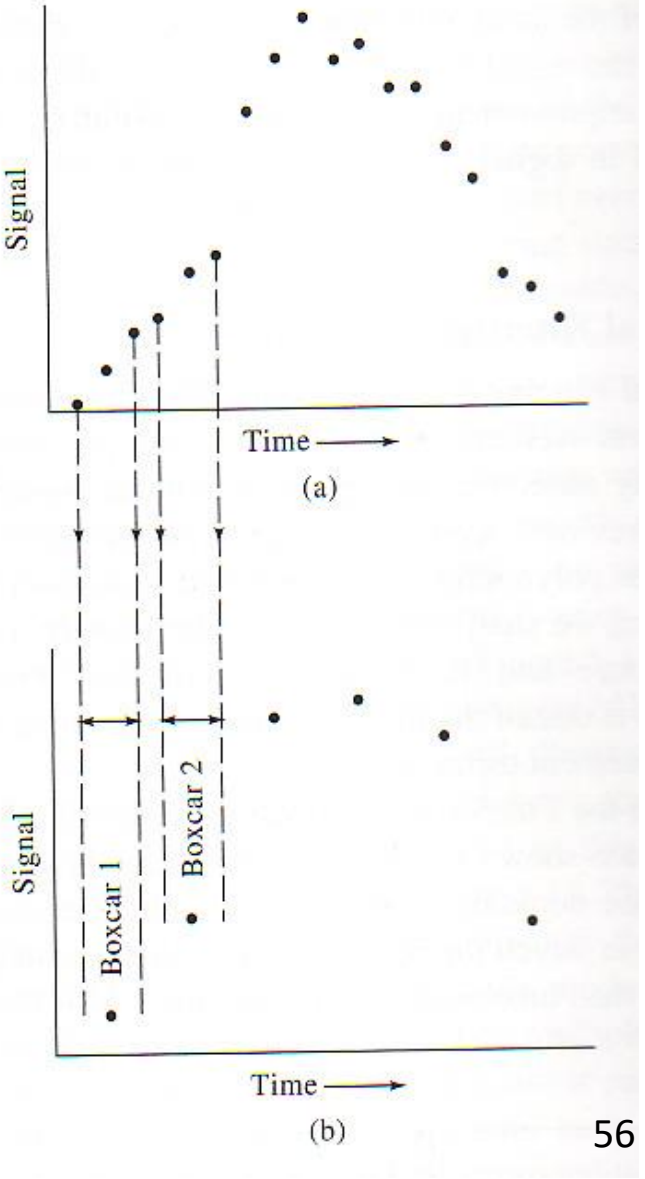
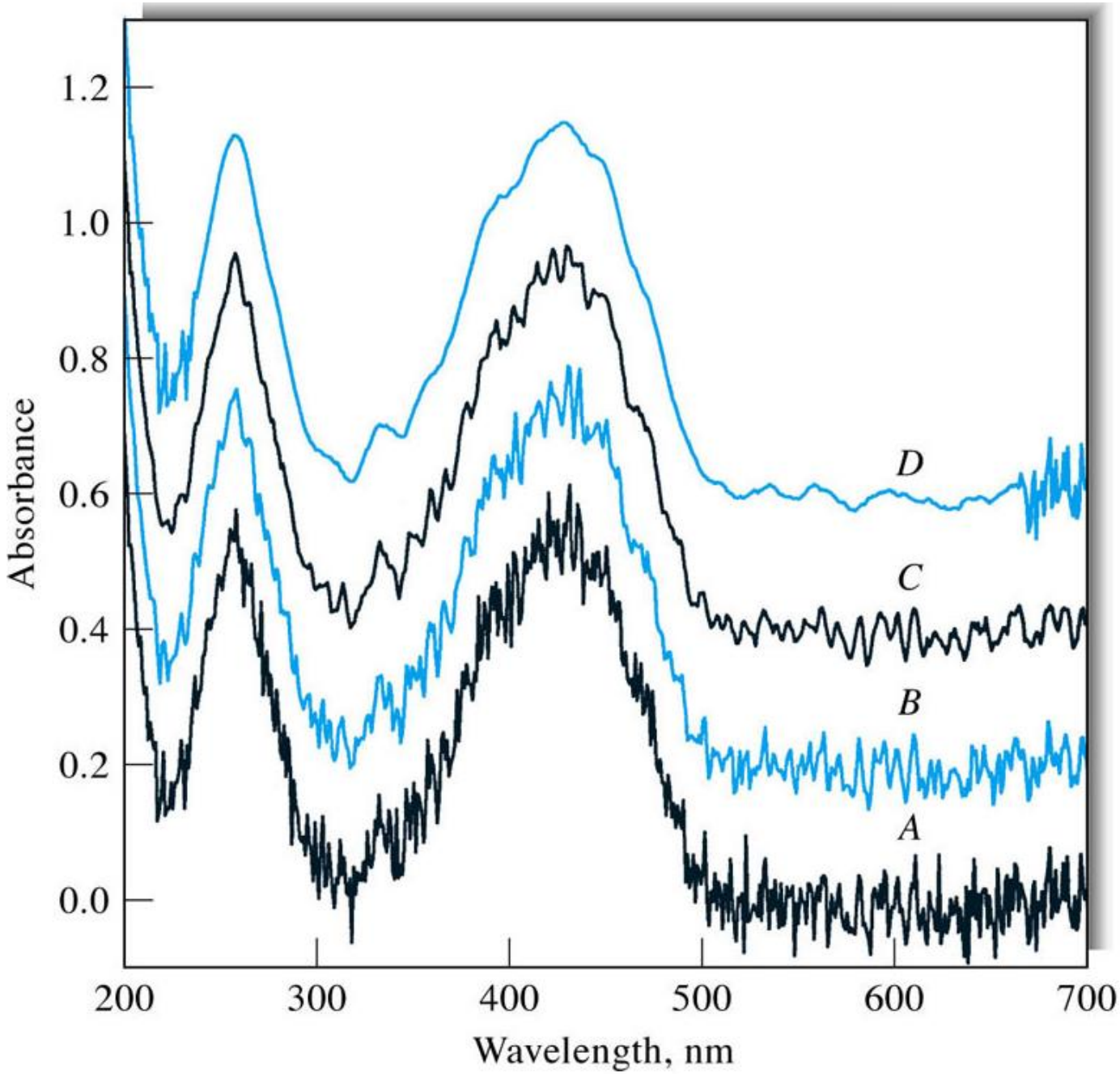


Figure 5-10 Effect of signal averaging. Note that the vertical scale is smaller as the number of scans increases. The signal-to-noise ratio is proportional to $\sqrt{n}$. Random fluctuations in the noise tend to cancel as the number of scans increases, but the signal accumulates; thus, *S/N* increases.




Absorbance
1.2
1.0
0.8
0.6
0.4
0.2
0.0
200
300
400
500
600
700
Wavelength, nm
D
C
B
A