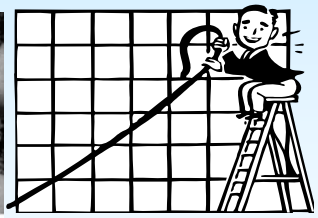
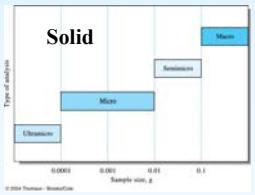
Chapter 8
Sampling, Standardization, and Calibration

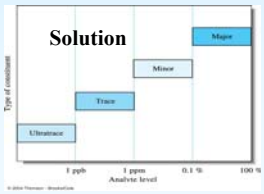


1

Classification of Analyses by Sample Size or Solution Constituent

	Solid Sample		Solution Sample	
Analytical techniques	**Sample size**	**Type of Analysis**	**Analytical level**	**Type of constituent**
Classical	100 mg	Macro-	> 1 mg/mL	Major-
	10 mg	Semi-micro-	1 mg/mL-1 μg/mL	Minor-
Modern	1 mg	Micro-	1 μg/mL-1 ng/mL	Trace-
	< 0.1 mg	Ultra-micro-	< 1 ng/mL	Ultra-trace-





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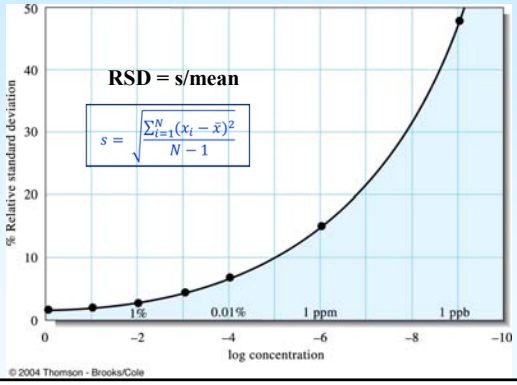
Interlaboratory Error vs. Analyte Concentration



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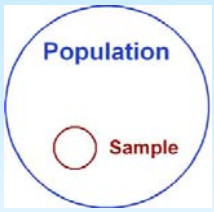
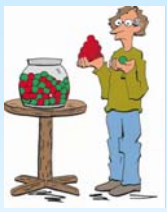
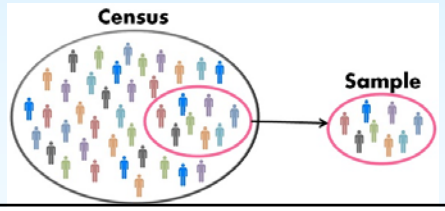
Sampling







Sample size
Biased sampling

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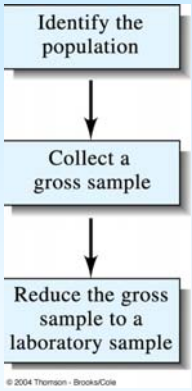
Steps in a Chemical Analysis

- Sampling—often the most difficult aspect of an analysis.
- Analyzing the Sample

e.g., Hg(II) in seawater
TNT in shooting ranges



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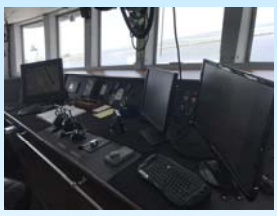
USM Marine Research Center: Research Vessels



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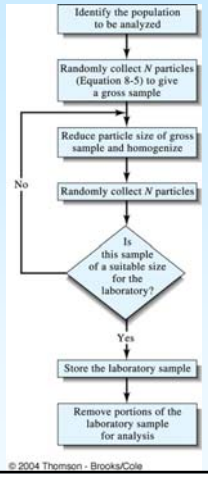
Steps in sampling a particular solid



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Analyzing the Sample

Step 1. Obtain a representative bulk sample.

Step 2. Extract from the bulk sample a smaller, homogeneous laboratory sample.

Step 3. Convert the laboratory sample into a form suitable for analysis, a process that usually involves dissolving the sample.

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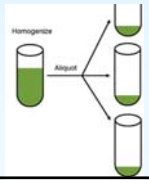
Analyzing the Sample

Step 4. Remove or *mask* species that will interfere with the chemical analysis.

Step 5. Measure the concentration of the analyte in several aliquots.

Step 6. Interpret your results and draw conclusions.

Aliquot: a portion of a larger whole, especially a sample taken for chemical analysis or other treatment.



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Standardization and Calibration

Every chemical analysis needs a way to relate the concentration or # of moles of analyte in solution to the observable property (mass, charge, current, voltage, pH, light intensity, temperature…) which is being measured.

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External Standard Calibration

- Use a series of known concentrations of standard solution to obtain measurable signals, and plot the signal intensity as a function of concentration. By comparing the signal obtained from unknown sample with the (standard) "calibration curve" (or "working curve"), the concentration of the unknown sample is obtained.
- Identical experimental conditions for standards and samples should be used.

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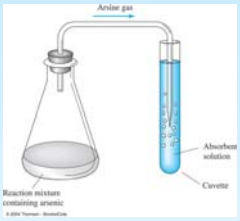
Determination of Arsenic Samples

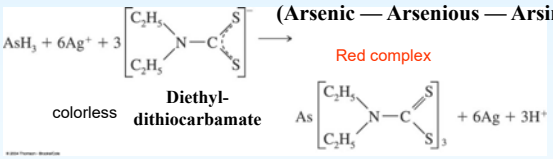
Herbicides may be used



$\xrightarrow{SnCl_2}$ $\xrightarrow{+Zn}$

$H_3AsO_4 \rightarrow H_3AsO_3 \rightarrow AsH_3$ (g)

(Arsenic — Arsenious — Arsine)



colorless

Diethyl-dithiocarbamate

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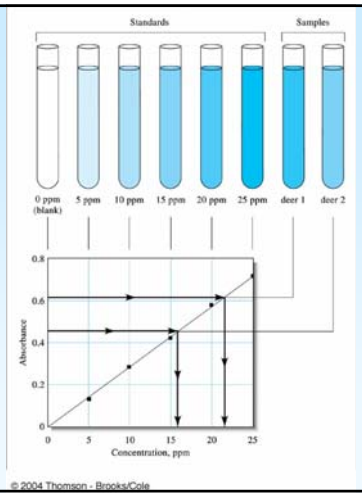
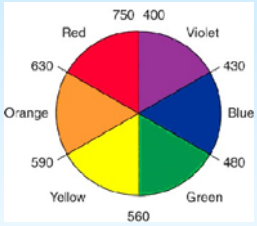


Color Wheel
Complementary Colors



ROY G. BIV

"Roy G. Biv is a colorful man"

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For many analytical techniques, we need to evaluate the response of the unknown sample against a set of standards (known quantities).

Example

I prepared 6 solutions with a known concentration of Cr^{6+} and added the necessary colouring agents. I then used a UV-vis spectrophotometer and measured the absorbance for each solution at a particular wavelength. The results are in the table below.

Erin Brockovich (Julia Roberts, 2000)
https://www.youtube.com/watch?v=ELzu636Xf6Y

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Concentration / $mg.l^{-1}$	Absorbance	Corrected absorbance
0	0.002	
1	0.078	
2	0.163	
4	0.297	
6	0.464	
8	0.600	

Note:
Corrected absorbance =
(absorbance of sample) – (absorbance of blank)

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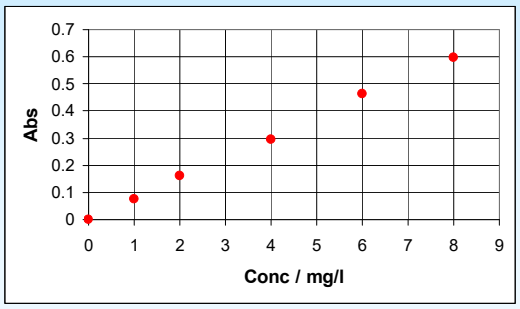
Calibration curve:



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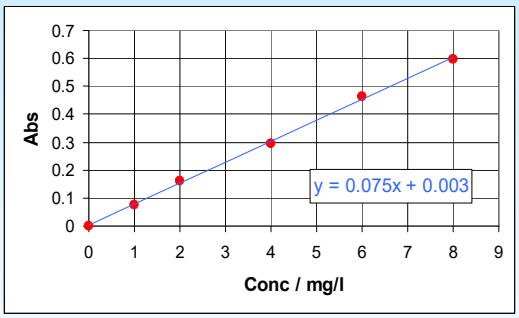
Fit best straight line:



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I then measured my sample to have an absorbance of 0.418 and the blank, 0.003. I can calculate the concentration using my calibration curve.

y = 0.0750x + 0.003

For my unknown:

absorbance = 0.418 – 0.003 = 0.415

Check on your calibration curve!!

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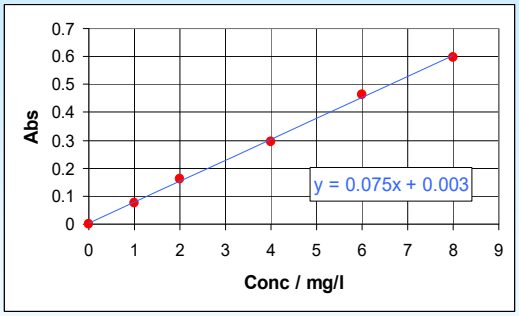
Absorbance = 0.415

Conc = 5.49 mg.l^{-1}



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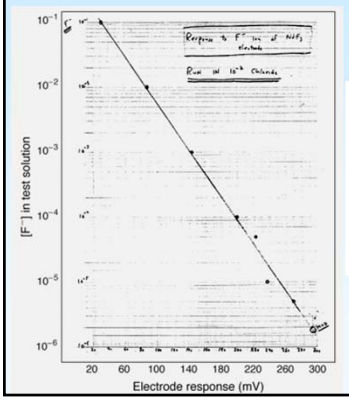
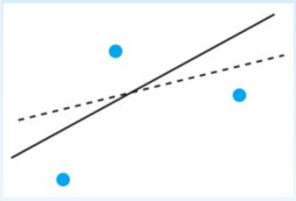
How do we find the best straight line to pass through the experimental points?





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METHOD OF LEAST SQUARES

Assume:

- **There is a linear relationship**
- **Errors in the y-values (measured values) are greater than the errors in the x-values.**
- **Uncertainties for all y-values are the same**

We try to minimize only the vertical deviations because we assume that the error in the y-values are greater than that in the x-values.

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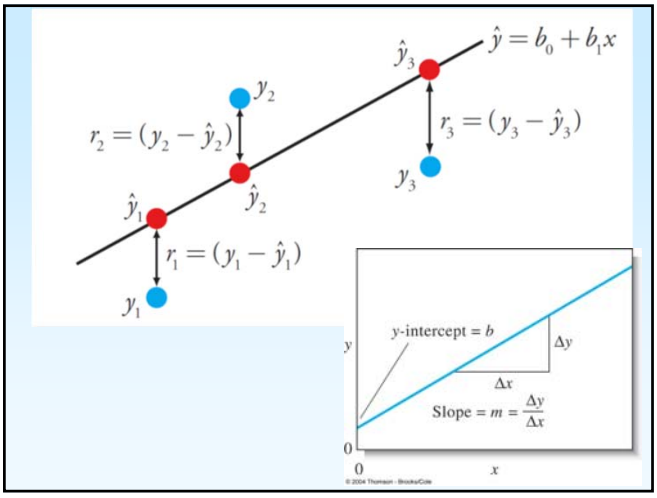

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Recall:

Equation of a straight line:

$y = mx + b$

where m = slope and b = y-intercept

We thus need to calculate m and b for a set of points.

Points = (x_i, y_i) for i = 1 to n

(n = total number of points)

$$m = \frac{n\sum(x_i y_i) - \sum x_i \sum y_i}{n\sum(x_i^2) - (\sum x_i)^2}$$

$$b = \frac{\sum(x_i^2)\sum y_i - \sum(x_i y_i)\sum x_i}{n\sum(x_i^2) - (\sum x_i)^2}$$

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The vertical deviation can be calculated as follows:

$d_i = (mx_i + b) - y_i$

Some deviations are positive (point lies above the curve) **and some are negative** (point lies below the curve).

Our aim → to reduce the deviations

⇒ square the values so that the sign does not play a role.

$d_i^2 = [(mx_i + b) - y_i)]^2$

Minimize → "least squares"

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Example **y = 0.075x + 0.003**

x_i	y_i	x_iy_i	x_i^2
0	0.000	0	0
1	0.076	0.076	1
2	0.161	0.322	4
4	0.295	1.180	16
6	0.462	2.772	36
8	0.598	4.784	64
Σx_i =	Σy_i =	Σx_iy_i =	$\Sigma(x_i^2)$ =

Slope: **y-intercept:**

$$m = \frac{n\sum(x_i y_i) - \sum x_i \sum y_i}{n\sum(x_i^2) - (\sum x_i)^2} \qquad b = \frac{\sum(x_i^2)\sum y_i - \sum(x_i y_i)\sum x_i}{n\sum(x_i^2) - (\sum x_i)^2}$$

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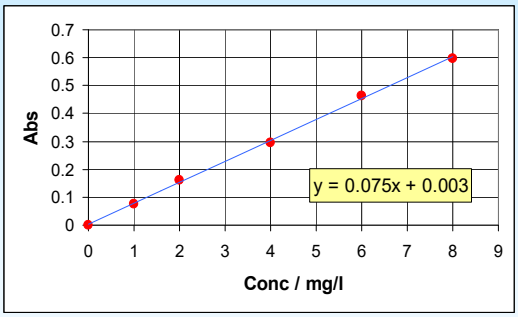
Linear Fit Using Excel Spreadsheet



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Matrix Effect

Matrix: Components of a sample other than the analyte of interest.

Matrix effect:

Some times certain components interfere in the analysis by either enhancing or depressing the analytical signal.

e.g., species activities are affected by the ionic strength.

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How do we circumvent the problem of matrix effects?

1. Internal standard method (a ref std added)
2. Internal standard addition method (same std)

Add a small volume of concentrated standard solution to a known volume of the unknown.

Assumption:

The matrix will have the same effect on the added analyte as it would on the original analyte in the sample

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Internal Standard

- Internal standard is a reference species, chemically and physically similar to analyte, that is added to samples, standards, and blanks.
- The ratio of the response of the (standard) analyte to that of the internal (reference) standard is plotted vs. the concentration of analyte.

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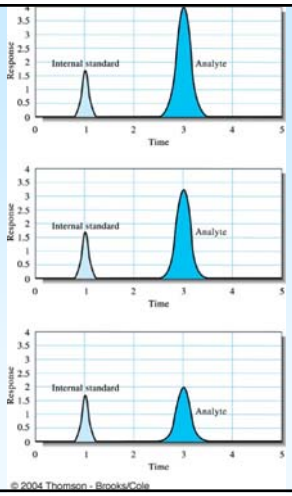

- A fixed amount of the internal standard is added.
- Internal standard is particularly helpful in dealing with variation in data at the instrumental level - things that would affect the sample and internal standard in the same way. e.g, experimental temperature, injection size changes.

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EXAMPLE 8-7

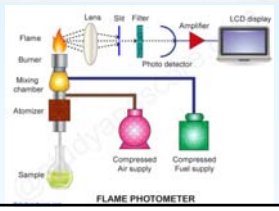
The intensities of flame emission lines can be influenced by a variety of instrumental factors, including flame temperature, flow rate of solution, and nebulizer efficiency. We can compensate for variations in these factors by using the internal standard method. Here, we add the same amount of internal standard to mixtures containing known amounts of the analyte and to the samples of unknown analyte concentration. We then take the ratio of the intensity of the analyte line to that of the internal standard. The internal standard should be absent in the sample to be analyzed.

In the flame emission determination of sodium, lithium is often added as an internal standard. The following data were obtained for solutions containing Na and 100 ppm Li.

reference

ppm Na	Na emission intensity	Li emission intensity (1000 ppm)
0.10	0.11	86
0.50	0.52	80
1.00	1.8	128
5.00	5.9	91
10.00	9.5	73
Unknown	4.4	95

(continued)



FLAME PHOTOMETER

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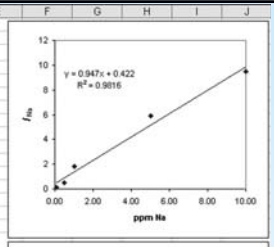
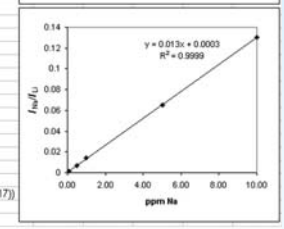
	A	B	C	D	E	F	G	H	I	J
1	**Method of internal standards for flame spectrometry**									
2	1000 ppm Li added as internal standard									
3	ppm Na	I_{Na}	I_{Li}	I_{Na}/I_{Li}						
4	0.10	0.11	86	0.001279						
5	0.50	0.52	80	0.0065						
6	1.00	1.8	128	0.014063						
7	5.00	5.9	91	0.064835						
8	10.00	9.5	73	0.130137						
9	Unknown	4.4	95	0.046316						
10	**Regression equation**									
11	Slope	0.012975								
12	Intercept	0.000285								
13	Concentration of unknown	3.54759								
14	**Error Analysis**									
15	Standard error in Y	0.000556								
16	N	5								
17	S_{xx}	71.148								
18	y bar (average ratio)	0.043363								
19	M	1								
20	Standard deviation in c	0.046925								
21	**Documentation**									
22	Cell D4=B4/C4									
23	Cell B11=SLOPE(D4:D8,A4:A8)									
24	Cell B12=INTERCEPT(D4:D8,A4:A8)									
25	Cell B13=(D9-B12)/B11									
26	Cell B15=STEYX(D4:D8,A4:A8)									
27	Cell B16=COUNT(A4:A8)									
28	Cell B17=DEVSQ(A4:A8)									
29	Cell B18=AVERAGE(D4:D8)									
30	Cell B19=enter no. of replicates									
31	Cell B20=B15/B11*SQRT(1/B19+1/B16+((D9-B18)^2)/((B11^2)*B17))									
32										
33										





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Internal Standard Addition Method

- Single-point standard addition method—a known amount of standard solution is added to one portion of the sample. The response before and after the addition are measured and used to obtain the analyte concentration.
- Multiple additions method are made to several portions of the sample.
- Assumes a linear response—should be always confirmed or the multiple additions methods used to check the linearity.

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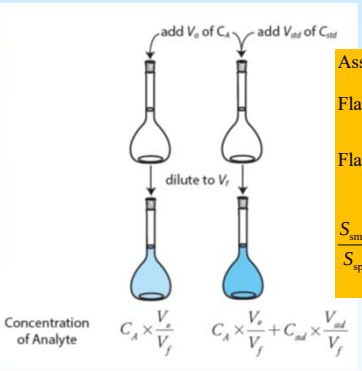
Single Standard Addition



Assuming : $S = k_A C_A$

Flask 1: $S_{smaple} = k_A (C_A \times \frac{V_0}{V_f})$

Flask 2: $S_{spike} = k_A (C_A \times \frac{V_0}{V_f} + C_{std} \times \frac{V_{std}}{V_f})$

$$\frac{S_{smaple}}{S_{spike}} = \frac{C_A \times \frac{V_0}{V_f}}{C_A \times \frac{V_0}{V_f} + C_{std} \times \frac{V_{std}}{V_f}} \Rightarrow C_A = ?$$

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Single-point addition Example:

Fe was analysed in a zinc electrolyte. The signal obtained from an atomic absorption spectroscopy (AAS) for Fe was 0.381 absorbance units. 5 ml of a 0.2 M Fe standard was added to 95 ml of the sample. The signal obtained was 0.805.

$\because$ (Beer's law) $A = kc; A_1 = kc_u, A_2 = kc_2$

$$\frac{A_1}{A_2} = \frac{c_u}{c_2} = \frac{c_u}{\frac{c_u v_u + c_s v_s}{v_u + v_s}} = \frac{c_u}{\frac{c_u v_u + c_s v_s}{v_t}} = \frac{c_u v_t}{c_u v_u + c_s v_s}$$

$$\therefore c_u = \frac{A_1 c_s v_s}{A_2 v_t - A_1 v_u} \quad = ? \text{ M} \qquad \text{(0.0086 M)}$$

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Note that when we add the 5 ml standard solution to the 95 ml sample solution, we are diluting the solutions.

Thus we need to take the DILUTION FACTOR into account.

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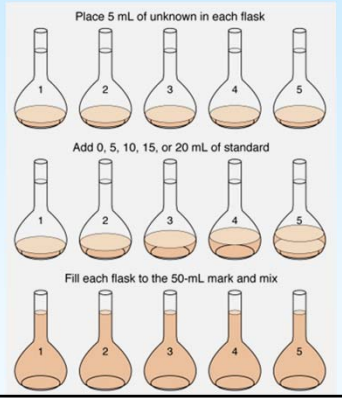
Internal multiple-point standard addition method
--How is this best done in practise?



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- The solutions in the above flasks all have the same concentration of the matrix.
- Analyze all solutions.
- Add a quantity of standard solution such that the signal is increased by about 1.5 to 3 times that for the original sample.

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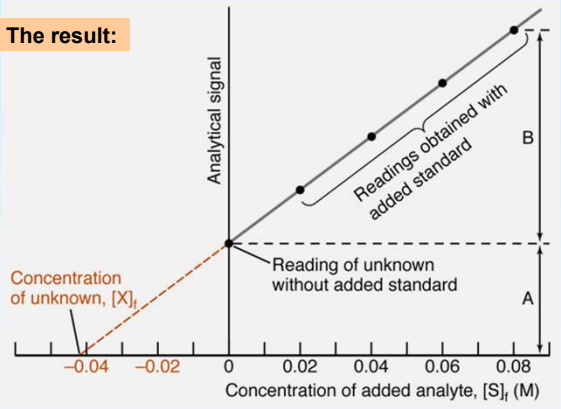
The result:



http://zimmer.csufresno.edu/~davidz/Chem106/StdAddn/StdAddn.html for details

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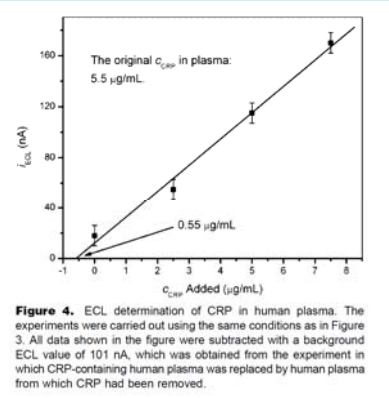

Figure 4. ECL determination of CRP in human plasma. The experiments were carried out using the same conditions as in Figure 3. All data shown in the figure were subtracted with a background ECL value of 101 nA, which was obtained from the experiment in which CRP-containing human plasma was replaced by human plasma from which CRP had been removed.

Miao, W.; Bard, A. J., Electrogenerated Chemiluminescence. 80. C-Reactive Protein Determination at High Amplification with $[Ru(bpy)_3]^{2+}$-Containing Microspheres. *Anal. Chem. 2004, 76 (23), 7109-7113.*

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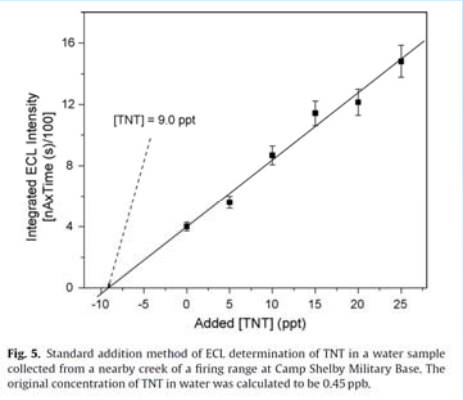

Fig. 5. Standard addition method of ECL determination of TNT in a water sample collected from a nearby creek of a firing range at Camp Shelby Military Base. The original concentration of TNT in water was calculated to be 0.45 ppb.

Pittman, T. L.; Thomson, B.; Miao, W., Ultrasensitive Detection of TNT in Soil, Water, Using Enhanced Electrogenerated Chemiluminescence. *Anal. Chim. Acta 2009, 632 (2), 197-202.*

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Figures of Merit for Analytical Methods

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Sensitivity

- Describes how much the observable changes with concentration

2 factors limiting sensitivity:

- slope of calibration curve
  - steeper slope, greater sensitivity
- reproducibility of measurements
  - equal slope, better reproducibility, greater sensitivity

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Detection Limit (Limit of Detection, LOD)

- The lowest concentration level that can be determined to be statistically different from the analyte blank.
- All instrumental methods have a degree of noise associated with the measurement → limits the amount of analyte that can be detected.

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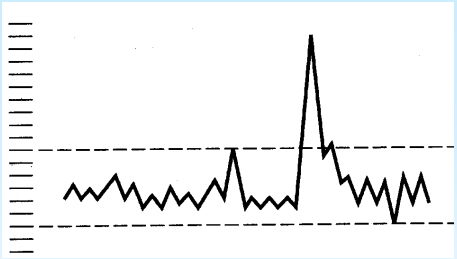

- Generally, the sample signal must be above 3x the standard deviation of the background signal

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Experimental Procedures to Obtain LOD

1. Prepare a sample whose concentration is ~1-5 times the estimated detection limit.
2. Measure the signal from n replicate samples ($n \geq 7$).
3. Compute the standard deviation (s) of the n measurements.
4. Measure the signal from n blanks (containing no analyte) and find the mean value, y_{blank}
5. **Signal detection limit:** $y_{dl} = y_{blank} + 3s$ ($n \geq 7$).
6. The corrected signal, $y_{sample} - y_{blank}$, is proportional to sample concentration

Calibration line: $y_{sample} - y_{blank} = m \times [sample]$

y_{sample}: signal observed for the sample and m is the slope of the linear calibration curve.

Minimum detectable concentration: LOD = $3s/m$

For accurate measurement: **Quantitation limit** = $10s/m$

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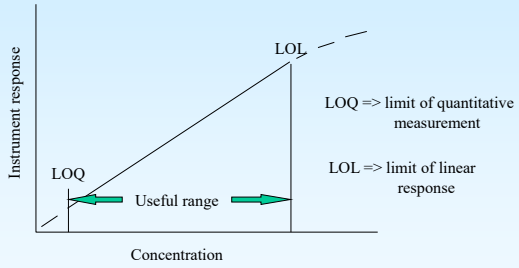
Applicable Concentration Range (Dynamic range)



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Selectivity

degree to which a method is free from interference by other species contained in the matrix

$$S = m_A c_A + m_B c_B + m_C c_C + S_{bl}$$

where S: analytical signal

c_A, c_B, c_C: concentrations of A, B, and C,

m_A, m_B, m_C: calibration sensitivities of A, B, and C, respectively, slope of calibration curve

S_{bl}: instrumental signal of blank

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Selectivity

$k_{B,A} = m_B/m_A$ and $k_{C,A} = m_C/m_A$

where $k_{B,A}$: selectivity coefficient for B with respect to A

$k_{C,A}$: selectivity coefficient for C with respect to A

yielding

$S = m_A(c_A + k_{B,A}c_B + k_{C,A}c_C) + S_{blank}$

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Chapter 8 Summary

- Analyses are classified by sample size as macro, semimicro, micro, and ultramicro.
- Analyses are classified by analyte level as major, minor, trace, and ultratrace.
- Samples are analyzed, but constituents or concentrations are determined.
- Calibration determines the relationship between the analytical response and the known analyte concentration. This plot is called a calibration curve.

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- "Least squares" is one mathematical method for performing regression analysis.
- An internal standard is a reference species, *chemically and physically* similar to the analyte added to samples/standards/blanks. The ratio of the response of the analyte to that of the internal standard is plotted vs. [analyte].
- "Standard additions" is used when it is difficult or impossible to duplicate the sample matrix.
- Figures of merits e.g., accuracy, precision, calibration sensitivity, detection limit, and linear dynamic range.

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