

<u>Chem 409 – Instrumental Analysis</u>

Course Objectives

- Students should acquire fundamental knowledge and skills in the following areas:
 - Modern analytical instrumental techniques; strengths and limitations, common elements
 - Practical applications of instrumental techniques
 - Principles of method validation
 - Data analysis applied to validation elements and outliers
 - Technical writing and presentations

Review of Syllabus, Schedule, Topics to be Covered, and Policies and Procedures

- Review materials posted in BB Syllabus Folder
- Handouts
- Questions / Areas for Clarification

- <u>Analytical Chemistry</u> → Scientific field involving the separation, identification, and quantitation of chemical components in a sample
 - Atoms, molecules
- **<u>Sample</u>** → Material containing an analyte of interest
 - May take many forms (solid, liquid, gas, other)
 - Contains other materials that can interfere with the analysis
 - Many types (chemical, biochemical, forensic, material, etc.)
- Qualitative analysis → Identification of an analyte by classical and instrumental methods
 - Composition, structure, intrinsic property
- <u>Quantitative analysis</u> → Determination of the concentration of an analyte by classical and instrumental methods
 - Concentration, mass

<u>Classical Methods</u>

- Wet chemical or physical analysis with visual observation
- May involve a separation or clean-up step prior to analysis
 - Examples: extraction, precipitation, filtration, distillation
- Do not involve instrumentation
- Examples (titrations, metals by flame, gravimetric)

Instrumental Methods

- Analyses using an instrument to measure a property intrinsic to the analyte of interest
- Typically involves a separation or clean-up step prior to analysis

So... what constitutes an instrument? So... what constitutes a measurable intrinsic property?

- **Instrumentation and Instrumental Analysis** \bullet **Basic Requirements:**
 - An energy source to interact with the analyte
 - A means of introducing the sample for analysis
 - A detector to measure the analyte's response
 - A means of converting the response to information usable to an analyst or researcher



Intrinsic Properties of Analytes

- Atomic and molecular energy levels
- Thermal transitions
- Elemental composition
- Particle size and morphology
- Electron transfer / conductivity
- Radioactivity
- Others
- <u>Detectors</u> measure either a change in energy impinging on the analyte or the response of the analyte to the impinging energy
 - Absorption of ultraviolet radiation by pyrene
 - Pyrene's fluorescence emission of visible radiation following UV absorption

Characteristic Properties	Instrumental Methods						
Emission of radiation	Emission spectroscopy (X-ray, UV, visible, electron, Auger); fluorescence, phosphorescence, and luminescence (X-ray, UV, and visible)						
Absorption of radiation	Spectrophotometry and photometry (X-ray, UV, visible, IR); photoacoustic spectroso nuclear magnetic resonance and electron spin resonance spectroscopy						
Scattering of radiation	Turbidimetry; nephelometry; Raman spectroscopy						
Refraction of radiation	Refractometry; interferometry						
Diffraction of radiation	X-ray and electron diffraction methods						
Rotation of radiation	Polarimetry; optical rotary dispersion; circular dichroism						
Electrical potential	Potentiometry; chronopotentiometry						
Electrical charge	Coulometry						
Electrical current	Amperometry; polarography						
Electrical resistance	Conductometry						
Mass	Gravimetry (quartz crystal microbalance)						
Mass-to-charge ratio	Mass spectrometry						
Rate of reaction	Kinetic methods						
Thermal characteristics	Thermal gravimetry and titrimetry; differential scanning calorimetry; differential thermal analyses; thermal conductometric methods						
Radioactivity	Activation and isotope dilution methods						

TABLE 1-1 Chemical and Physical Properties Used in Instrumental Methods

Detectors

- A device that senses a change in one of the variables in its environment
- Electromagnetic radiation, electrical charge, particulates, molecules, temperature, mass, etc.

<u>Transducers</u>

- A device that coverts information in the nonelectrical domain to information in the electrical domain and the converse
- Information can be encoded into different electrical domains
 - Amplitude (magnitude), time (frequency, period), digital (binary; ON-OFF, HI-LO)

<u>Transducers</u>

Convert the analyte's response to information in a usable format for an analyst or researcher

– Three types: Input – Conversion – Output



See table 1-2 in text along with Data Domain discussion

Instrumental Analysis Methods

Considerations for development and/or the selection of an established method for analysis

- What needs to be measured and why?
- Qualitative or quantitative results needed?
- What level of quantification and detection is needed?
- What level of accuracy and precision is needed?
- How much sample is available?
- What sample preparation and instrumental techniques are available?
- What else is in the sample? Potential interferences?
- How many samples will be analyzed and how often?
- What are the pre-requisite skills and training requirements needed for analysis method selected?

Instrumental Analysis Methods

Basic elements once technique or method selected



<u>Calibration</u>

- 1. Method calibration establishes relationship of analytical signal to analyte concentration
- 2. Instrument calibration establishes accuracy of critical equipment (e.g. balances, pipettes, instrument)

Lecture 2

- <u>Calibration Methods for Quantitative Analysis</u>
 - Univariate methods
 - One signal is monitored that is related to one concentration
 - Types of standard calibration univariate methods
 - –External standard
 - –Internal standard
 - -Standard addition
 - Multivariate methods
 - Several different signals are monitored that are related to one concentration

Types of Error

Determinate or systematic error

- Cause may be identified and eliminated or minimized
- Error associated with the analytical procedure and/or instrument(s)
- Is constant, proportional or multiplicative, (+ / –)
- Indeterminate or random error
 - Can not be identified, avoided, or corrected
 - Occurs randomly and has a random distribution, (+ / –)
- The method of calibration for quantitative analysis is influenced by the type of error present and strategies to minimize the error to achieve the accuracy, precision, and sensitivity requirements

- **External Standard Calibration** (non-Matrix & Matrix Matched)
 - Strength: Simplicity and easy of use in cases where sufficient precision and accuracy can be achieved.
 - Limitation: Matrix matching requires securing a reliable source and quantity of placebo sample matrix. Placebo = a sample that does not contain the analyte of interest.



- Internal Standard Calibration (non-Matrix & Matrix Matched)
 - Strength: Compensates for several types of systematic and random error associated with instrument and method-related variation.
 - Limitation: Identification of a suitable internal standard material. Increased method complexity, time and cost.

Experimental

- Include a known concentration of a related compound in the standards and the unknown(s) that gives a similar but distinct response from the analyte
- Measure S_A and S_{IS}
- Plot S_A/S_{IS} vs. c_A

 S_A = signal for analyte S_{IS} = signal for internal standard C_A = concentration for analyte

Internal Standard Calibration

	A	В	С	D	E.	F	G	Н		J	К		
1	Method of internal standard	ds for flame	e spectrom	etry	T	External Std. Diat							
2	1000 ppm Li added as intern	al standard				12							
3	Na conc., ppm	/ _{Na}	/ _{Li}	/ _{Na} /I _{Li}									
4	0.10	0.11	86	0.001279		10	- 0.047						
5	0.50	0.52	80	0.0065			y = 0.947x	0946		/			
6	1.00	1.8	128	0.014063		8	R*=0.9816						
7	5.00	5.9	91	0.064835					/				
8	10.00	9.5	73	0.130137		2 6		•					
9	Unknown	4.4	95	0.046316									
10	Regression equation					4							
11	Slope	0.012975	5			2					0		
12	Intercept	0.000285									2		
13	Concentration of unknown	3.54759				0							
14	Error Analysis					0.00	2.00	4.00	6.00	8.00	10.00		
15	Standard error in Y	0.000556					Na conc. nom						
16	N	5						na oon	, ppm				
17	S _{xx}	71.148											
18	y bar (average ratio)	0.043363	8			35124	Internal Std. Plot						
19	Μ	1			i i i	0.14							
20	Standard deviation in c	0.046925				0.12							
21	Documentation						$y = 0.0^{4}$	13x + 0.0003					
22	Cell D4=B4/C4					0.1	$R^2 = 0.9999$						
23	Cell B11=SLOPE(D4:D8,A4:A8)				→ 0.08				106				
24	Cell B12=INTERCEPT(D4:D8,A4:A8)												
25	Cell B13=(D9-B12)/B11	10				- 0.06							
26	Cell B15=STEYX(D4:D8,A4:/	A8)				0.04							
27	Cell B16=COUNT(A4:A8)					00703380 24204264							
28	Cell B17=DEVSQ(A4:A8)					0.02	*						
29	Cell B18=AVERAGE(D4:D8)					0							
30	30 Cell B19=enter no. of replicates					0.00	2.00	4.00	6.00	8.00	10.00		
31	Cell B20=B15/B11*SQRT(1/B19+1/B16+((D9-B18)^2)/((B11^2)*B17))									100000000			
32			5						ion hhim				
33						L			0	í	<u> </u>		

Method of Standard Addition

- Strength: Useful in cases where significant sample matrix effects are present and a placebo sample matrix for external standard calibration is unavailable.
- Limitation: Increased method complexity, time and cost.

Experimental

- For a single unknown, prepare the same amount of sample multiple times (e.g. n = 4 aliquots)
- Add different volumes of the standard to each of the unknown sample aliquots
- Measure $S_{\rm A}$, Plot $S_{\rm A}$ vs. $c_{\rm A}$
- Extrapolate line to X-intercept; $(V_s)_0$
- $(V_{s})_{0}$ is the volume of the standard equivalent to the amount of analyte in the unknown sample

Method of Standard Addition



Performance Characteristics derived from Calibration Curves



- Calibration Sensitivity
- Dynamic Range
 - Theory
 - Practice
- Limit of Detection
- Limit of Quantitation

Normal or Gaussian Distribution

- Characteristic of data when only random error is present
- Spread of the distribution = standard deviation of mean (σ)
- Larger the error associated with a measurement, larger $\boldsymbol{\sigma}$

• LOD and LOQ in terms of σ

– % of measurements that within $\boldsymbol{\sigma}$



Limit of Detection (LOD)

• Minimum detectable signal - S_m

 $S_{m} = \overline{S}_{blank} + ks_{blank}$ s_{blank} - standard deviation of the blank k = 3, typically

 Minimum detectable concentration (LOD) - c_m

$$c_m = \frac{S_m - \overline{S}_{blank}}{m}$$



LOD

LOQ



Minimum quantifiable concentration (LOQ) - c_q

$$c_q = \frac{S_q - \overline{S}_{blank}}{m}$$

<u>Selectivity</u>

- Extent to which the response of the analyte is free from interference from other species in the sample
 - High selectivity method = free from interference
 - Low selectivity method = significant interference present
- Selectivity coefficient ($k_{B,A} = m_B/m_A$); m = slope

$$-S = m_A c_A + m_B c_B + S_{blank}$$

– What's S?

- Rearranging:
$$(k_{B,A} = m_B/m_A) \rightarrow m_B = k_{B,A} m_A$$

- Substitution: $S = m_A (c_A + k_{B,A} c_B) + S_{blank}$

<u>Selectivity</u>

Scenarios

 $m_{B} = 0; S_{blank} = 0$ $m_{B} = 0; S_{blank} = 0.1$

 $m_A = 0.07, m_B = 0.007$ $m_A = 0.07, m_B = 0.7$

 $m_A = 0.07, m_B = -0.007$ $m_A = 0.07, m_B = -0.7$ $S = m_A (c_A + k_{B,A} c_B) + S_{blank}$ Selectivity coefficient (k_{B,A} = m_B/m_A)



<u>Selectivity (Summary)</u>

- <u>Scenarios (response for species B relative to analyte A)</u>

- $k_{B,A} = 0$ method is highly selective
- k_{B,A} < 0 species B causes a depression in the analytical signal for A (concentration of A is underestimated)
- $k_{B,A} > 0$ signal enhancement; species B causes the concentration of A to be overestimated

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