

Chem 310 Lecture Notes

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Overview of the course:

This course is taken mainly by chemistry and forensic science students. Many of you will use instruments for chemical analyses in lab. settings. Some of you will go into careers (medicine, pharmacology, forensic science, environmental monitoring & remediation) which requires a working knowledge of instrumental analysis. The most widely used instrumental methods in these career paths are separation methods (gas & liquid chromatography) and mass spectrometry (often joined with chromatography in one instrument). The next tier of importance includes UV-VIS absorption spectroscopy, IR spectroscopy, fluorescence spectroscopy, atomic analysis methods (absorption and emission), and potentiometry. All of these methods will be covered in this course.

A problem arises with the choice of which more specialized methods to cover. In this course, we will discuss Raman spectroscopy (of growing importance in both pharmacology and forensic science) and voltammetry methods. Some of the reasons for covering voltammetry are the unique information that it provides (oxidation state), its application as a selective detector for liquid chromatography and its importance in medical research (e.g., monitoring neurotransmitter release and uptake in brains).

The order of topics (see the syllabus) is designed to provide essential information as it is needed. Consequently, spectroscopic methods are covered first and electrochemical methods second because these methods are used as detectors in separation science methods. Mass spectrometry follows separation science methods because it is easier to discuss hyphenated methods (e.g., gas chromatography-mass spectrometry or GC-MS) after discussing the separation methods. If there is time, the section on capillary electrophoresis, an increasingly important separation method, will be covered.

In order to allow discussion of many methods, there will be material in the lecture notes which will not be covered in lecture but for which you will be responsible. These will be clearly indicated. At the end of each section, there is a brief review of the important definitions, concepts and calculations on which you will be tested. A few topics are left in which will not appear in lecture or on tests. They are for your benefit.

Reading assignments are from Harris, *Quantitative Chemical Analysis*, 6th edition. Additional reading assignments are from Skoog, Holler and Nieman, *Principles of Instrumental Analysis*, 5th edition. The SH&N reading assignments are NOT required; they are for the instructor's benefit, since this course was originally tailored to that textbook.

Quick overview: Topics I, II, III and IV are relevant to all instrumental methods.

Reading: Harris, ch. 0, pp. 1-9; ch. 3, pp. 49-51; ch. 4, pp. 61-63; ch. 5, pp. 80-92; ch. 29, pp. 726-729
SH&N, ch. 1, pp. 1-3, 11-18.

I. DEFINITIONS.

Analyte - the substance being identified or quantified.

Sample - the mixture containing the analyte. Also known as the matrix.

Qualitative analysis - identification of the analyte.

Quantitative analysis - measurement of the amount or concentration of the analyte in the sample.

Signal - the output of the instrument (usually a voltage or a readout).

Blank Signal - the measured signal for a sample containing no analyte (the sample should be similar to a sample containing the analyte)

For most instrumental methods (exception: potentiometry), the signal is linear with respect to the concentration of the analyte over a range of concentrations:

$$S = mC + S_{bl}$$

where C = conc. of analyte; S = signal of instrument; m = sensitivity; S_{bl} = blank signal. The units of m depend on the instrument, but include reciprocal concentration.

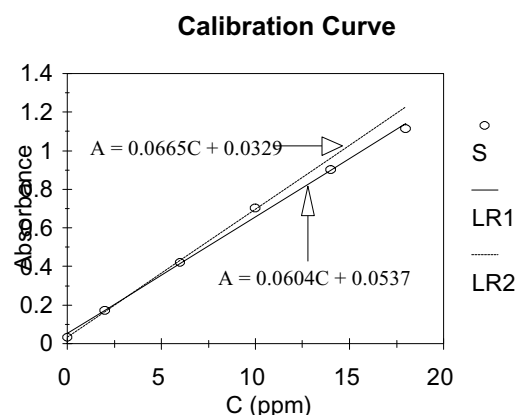
A standard (a.k.a. control) is a sample with known conc. of analyte which is otherwise similar to composition of unknown samples. A blank is one type of a standard. The words “standards” and “blanks” often refer to the signals generated by these types of samples.

The standard method of checking the above equation and defining the sensitivity of an instrumental method is to obtain a calibration curve - a plot of signal vs conc. for a set of standards. Calibration curves are often nonlinear at high and low concentrations, and linear at intermediate concentrations. The linear part of plot is the dynamic range. Often linear regression (method of least squares) is used to find the best straight line through experimental data points. **The slope of the linear part is the sensitivity m .**

Ex. Absorption spectroscopy

<u>C (ppm)</u>	<u>A (absorbance)</u>
0.00	0.031
2.00	0.173
6.00	0.422
10.00	0.702
14.00	0.901
18.00	1.113
[C?]	0.501

The first row is the measurement of the blank; the next 5 rows are data obtained from standards; the last row is an unknown. Questions: What is the linear dynamic range? What is the sensitivity? What is [C?]?



To find the linear dynamic range, plot the data and inspect it for a linear domain. In the preceding graph, the entire data set appears to be roughly linear. First approximation: the linear dynamic range is 0-18 ppm. Fitting all data to a linear regression line (LR1) yields a sensitivity of 0.0604 ppm^{-1} (slope of the LR line). However, closer inspection reveals that the data points show curvature. In particular, the highest 2 data points appear to be lower than expected based on the other data points. Second approximation: the linear dynamic range is 0-10 ppm. Fitting the first 4 data points to a linear regression line (LR2) produces a much better fit of data to the LR line. The sensitivity is 0.0665 ppm^{-1} . To calculate [C?], invert the equation:

$$A = 0.501 = 0.0665[C?] + 0.0329; [C?] = (0.501 - 0.0329)/0.0665 = 7.04 \text{ ppm}$$

Variation: Often, the blank signal is subtracted from all other data signals. The graph of the blank-corrected data ($S - S_{bl}$) should have an intercept near zero; i.e. $S_{corr} = mC$.

Note: on tests, be able to calculate the sensitivity given just two data sets. You can use linear regression, or set up two equations in two unknowns:

Equations: $S_1 = mC_1 + S_{bl}$ and $S_2 = mC_2 + S_{bl}$ (solve for m and S_{bl})

If the signal of the unknown falls between the two data sets, then you can use linear interpolation to calculate the concentration of the unknown.

Review:

Definitions: analyte; sample; blank; standard; sensitivity; dynamic range;

Calculations: Given calibration data, calculate sensitivity and the concentration of an analyte.

II. STATISTICS OF MEASUREMENT.

Precision - reproducibility of replicate measurements on a single sample.

Accuracy - agreement between measured conc. and true conc. (often not known).

Error - actual difference between measured conc. and true conc.

Ex. Target shooting.

Two types of error:

1. Random error (indeterminate error) - unpredictable and non-correctable changes in signal for replicate measurements.
2. Systematic error (determinate error)- predictable and usually correctable changes in signal from true value. Also known as bias.

Random error affects precision, systematic error affects accuracy.

Measurement of precision: make N replicate measurements ($x_1, x_2, \dots x_N$) on the same sample. x can be the signal S or the calculated conc. C .

$$\text{mean} = \langle x \rangle = (\sum x_i)/N$$

$$s \text{ (estimated standard deviation)} = [(\sum (x_i - \langle x \rangle)^2)/f]^{1/2}$$

where $f = \# \text{ degrees of freedom} = N-1$ for a single set of n measurements. s is the measure of precision.
RSD = relative standard deviation = $s/\langle x \rangle$ (often expressed as a percentage, which is the coefficient of variation); variance = s^2 . Note: You will not be tested on these formulas. Standard deviation can be calculated with built-in functions on most scientific calculators and spreadsheets.

The limit of detection (LOD) is the conc. at which one is 95% confident the analyte is present in the sample. The LOD is affected by the precision of the measurements and by the magnitude of the blanks. From multiple measurements of blanks, determine the standard deviation of the blank signal s_{bl} .

Then $LOD = 3s_{bl}/m$ where m is the sensitivity.

However, precision at the LOD is poor. The limit of quantitation LOQ is the smallest conc. at which a reasonable precision can be obtained (as expressed by s). The LOQ is obtained by substituting 10 for 3 in the above equation; i.e., $LOQ = 10s_{bl}/m$.

Ex. In the earlier example of absorption spectroscopy, the standard deviation of the blank absorbance for 10 measurements was 0.0079. What is the LOD and LOQ?

$$s_{bl} = 0.0079; m = 0.0665 \text{ ppm}^{-1}; LOD = 3(0.0079)/(0.0665 \text{ ppm}^{-1}) = 0.36 \text{ ppm}$$
$$LOQ = 10(0.0079)/(0.0665 \text{ ppm}^{-1}) = 1.2 \text{ ppm}$$

Review:

Definitions: precision; accuracy; random and systematic error; limit of detection (LOD); limit of quantitation (LOQ)

Calculations: From replicate measurements of one sample, calculate the mean, standard deviation, RSD and S/N. Given sensitivity and the standard deviation of the blank, calculate LOD and LOQ.

III. MEASUREMENT METHODS.

A major problem in measurement of analyte conc. in samples is the matrix effect. The matrix of the sample interferes with the measurement. For example, a solid sample can be non-uniform. Then light is scattered by the sample, and the measured absorbance is higher than the true absorbance.

Elements, ions, or compounds that specifically interfere with the measurement of a particular analyte are called interferences.

Techniques for reducing matrix effects include:

1. Matrix substitution - dissolving sample into liquid or gas solution, grinding sample with KBr powder.
2. Separation - using chromatography, solvent extraction, etc. to isolate analyte from complex matrix.
3. Preconcentration - collecting the analyte from sample into a much smaller volume to raise its concentration.
4. Derivatization - chemically modifying the analyte to improve volatility, light absorption, complex formation, etc., so that the instrument can more easily measure concentration.
5. Masking - modifying interferences so that they are no longer detected by the instrument.

An absolute method requires no calibration to calculate concentration from the output of the instrument. The sensitivity can be obtained from theoretical equations. Example: coulometry or coulometric titration.

All other methods require calibration to determine the sensitivity. There are 3 procedures for extracting concentration from instrument output.

- (a) Calibration curve (a.k.a. working curve) (see section I) is a plot of signal S vs. conc. C created by measuring a series of standards for a well-defined set of conditions. It is the best method if matrix effects are small and are independent of conc. of the analyte. The least error in measured conc. occurs when the sample signal is bracketed by standards (higher and lower signals for standards).
- (b) Standard addition (a.k.a. spiking) consists of at least three steps. First, measure the signal from the sample; second, add a known conc. of analyte (the spike) to sample; third, remeasure the

signal. With only one spike, you must assume linear response of the signal with conc. Additional spiking improves the precision and proves that the signal is linear with conc. Standard addition is especially useful when matrix effects are severe or when concentrations are near the detection limit. For the best precision, the first spike should at least double the analyte conc. in the sample.

For this course, be able to calculate via equations the conc. of an analyte given a single spike. Assumption: the data are blank-corrected (blank signal subtracted). There are 2 variations to this problem. Each involves writing 2 equations in 2 unknowns. The differences are the dilution factors (ratio of volumes).

Variation 1: A known volume of sample (V_x) is placed in one flask and diluted to the mark (V_t). The same volume of sample plus a known volume (V_s) of a standard (conc. C_s) is placed in a 2nd flask and diluted to the mark. The signal is measured on both solutions. Calculate C_x .

Equations: $S_1 = mC_x(V_x/V_t)$ and $S_2 = m(C_x(V_x/V_t) + C_s(V_s/V_t))$

Variation 2: The sample of known volume (V_x) is measured. To the sample is added a known volume (V_s) of a standard (C_s) and the measurement is repeated. Calculate C_x .

Equations: $S_1 = mC_x$ and $S_2 = m(C_x V_x/(V_s + V_x) + C_s V_s/(V_s + V_x))$

Examples will be given in the first homework.

- (c) Internal standard is a substance added to all samples, blanks and standards so that its concentration is fixed and known. The signal due to the internal standard (S_{is}) is measured at nearly the same time as the signal due to the analyte. The ratio S/S_{is} is plotted vs conc. of standards as in calibration curves above. This method is useful when the sensitivity of the instrumental method fluctuates or drifts with time, and when matrix effects are severe.

Review:

Definitions: matrix effects.

Concepts: What are the 5 techniques for reducing matrix effects? What is an absolute method? What are calibration curves? What is standard addition?

Calculations: Calculate concentration of an analyte from calibration curve data or standard addition data for any method for which the signal is linear in concentration; be able to state any necessary assumptions needed to do the calculations.

IV. INSTRUMENTAL NOISE.

Reading: Harris, ch. 20, pp. 487-488

SH&N, ch. 5, pp. 99-108 (omit difference and instrumentation amplifiers)

Assumptions: the instrument signal is monitored with time, and the signal is converted to the digital domain. The resulting binary numbers correspond to a specified voltage range of the instrument signal.

Most instruments these days convert the analog signal (a voltage or a current) to a digital signal using an ADC (analog-to-digital converter). The ADC samples the signal over a short time and convert it to a binary number. The output is a string of binary numbers representing the signal at evenly spaced intervals of time. Resolution and the minimum noise is determined by the # of bits of the ADC.

Ex. a 12-bit ADC has a resolution of $1 \text{ in } 2^{12} = 1 \text{ in } 4096$; consequently, the resolution and minimum noise (and RSD) cannot be smaller than 1 part in 4096.

Ex. A 12-bit ADC will generate binary numbers between 0 and 4095. This represents a signal between -5 and $+5 \text{ V}$. What is the resolution of the transformed signal? What is its minimum noise?

Binary ADC output	0	...	4095
Transformed input	-5	...	+5 V

The input range is $\text{max.} - \text{min.}$, i.e. $\{(+5 \text{ V}) - (-5 \text{ V})\} = 10 \text{ V}$. This range is divided into 4096 parts by the ADC, so the resolution of the signal is $(10 \text{ V})/4096 = 0.0024 \text{ V}$ or 2.4 mV . The minimum noise is the same as the resolution (2.4 mV).

The sampling frequency f_s of an ADC is the number of times per second the ADC samples the analog signal and converts it to a binary number. The reciprocal of sampling frequency is the sampling period τ_s .

For quantitative analysis, usually a sample with a constant analyte conc. is measured for a period of time. The signal should be constant but is not, due to noise. Example: $\rightarrow$

The data points are obtained at a frequency of 100 Hz (sampling period $\tau = 0.01 \text{ s}$).

For qualitative analysis, usually some property of the sample is measured as a function of a variable (e.g., wavelength of light) which changes linearly with time. Ex. an absorption spectrum with a linear wavelength scan ($\lambda \propto t$). $\rightarrow$
($f = 100 \text{ Hz}$, $\tau = 0.01 \text{ s}$)

The data contain noise (random fluctuations in signal). Where the signal should be constant (quant. anal. figure), noise is defined quantitatively as the standard deviation of the signal:

$$s = [(\sum(S_i - \langle S \rangle)^2)/f]^{1/2}$$

Ex. the noise in the Quant. Anal. Signal figure is 1.14. The mean value is 10.17 (50 points).

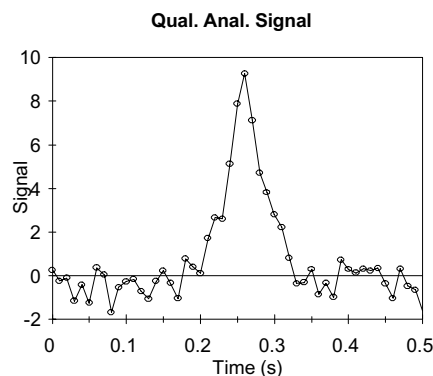
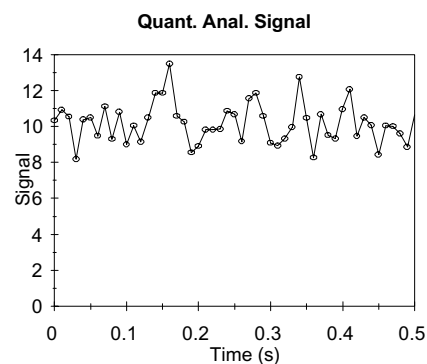
Define signal-to-noise (S/N) = $\langle S \rangle / s = 1/(\text{RSD})$
(a useful measure for data or instrument performance; higher S/N is desirable)

Ex. S/N in the Quant. Anal. figure = $10.17/1.14 = 8.92$

Where the signal is not constant (Qual. Anal. Figure), noise is obtained as the standard deviation from a region of relatively constant signal. Ex. the noise in the Quant. Anal. Signal figure between 0 and 0.1 s is 0.65.

S/N is usually defined for a peak value. Ex. S/N at the peak in the Qual. Anal. figure = $9.3/0.65 = 14$.

Types of instrument noise:



1. Environmental noise - includes power line noise at 60 Hz, harmonics (120 Hz, 180 Hz, ...) and sub-harmonics (30 Hz).
2. Johnson noise - random fluctuations of electrons in a conductor. "White" noise (present at all frequencies). Voltage noise proportional to square root of temperature (in Kelvin).
3. Shot noise - random variations of charged particles crossing an interface (present in most light detectors and diodes). "White" noise; current noise proportional to square root of current.
4. Flicker noise - origin not always clear. "Pink" noise; amplitude increases as frequency decreases. Includes drift - slow changes in instrument baseline and sensitivity.

Strategies to enhance S/N: Either enhance signal (discussed for various instrumental methods) or decrease the noise.

Methods for decreasing noise:

1. Shield the experiment, i.e., wrap it in a metal box connected to instrument ground. Removes noise induced by electrical fields (power line noise).
2. Cool the detector (frequently used for light detectors). Reduces Johnson noise.
3. Synchronous detection - modulate the "signal" (e.g. light beam, electrical voltage) at a fixed frequency. The amplitude of the periodic "signal" is changed by sample. Using special electronic equipment, isolate the signal at the fixed frequency and extract amplitude information. Reduces all types of noise, especially flicker noise.
4. Digitize the signal and use computer methods to decrease noise.
 - (a) For a DC signal (quant. anal.), digitize the signal N times and add the numbers in computer memory. Signal and baseline increase N times, but random noise only increases $\sqrt{N}$ times, so S/N increases by a factor of $\sqrt{N}$.

Ex. The first 5 data points of a quantitative measurement are: 5.55, 5.41, 6.26, 6.09, 5.48. Calculate the S/N. How many additional measurements are needed to increase the S/N to 30?

$$\text{mean} = (5.55 + 5.41 + 6.26 + 6.09 + 5.48)/5 = 5.76; \quad s = 0.39; \quad S/N = 5.76/0.39 = 15$$

$$S/N = k\sqrt{N}; \quad k = (S/N)/(\sqrt{N}) = 15/\sqrt{5} = 30/\sqrt{N}; \quad \sqrt{N} = (30/15)\sqrt{5}; \quad N = (4)(5) = 20$$

- (b) For a time-dependent signal (qual. anal. spectra), use ensemble averaging. Trigger the measurement repeatedly, digitize the signal at fixed time intervals, and add the binary numbers into separate memory locations, one location for each timer interval after the trigger. Alternately, use a multichannel detector (found mainly in optical spectroscopy) with each channel being digitized and stored in separate memory locations. Again S/N improves by the factor of $\sqrt{N}$.

Review

Definitions: signal-to-noise.

Concepts: What are the 4 types of instrument noise? What strategies can be used to enhance S/N?

Calculations: Calculate the change in S/N with N (# of measurements averaged).

Overview: Topics V, VI, VII, VIII, and IX discuss concepts relevant to most optical instruments (spectrometers) and components that appear in most optical instruments.

V. BASIC OPTICS. (for some neat instruction on optics on the WEB, go to <http://www.micro.magnet.fsu.edu/optics/tutorials/index.html>)

This material will NOT be covered in lecture. You are responsible for reading the material and understanding it, most of which is covered in basic physics courses.

Reading: Harris,

SH&N, ch. 6, pp. 116-130 (review of electromagnetic wave behavior).

You are assumed to know about refraction, diffraction, reflection, coherent radiation, index of refraction, polarization, scattering, and the photoelectric effect. This section applies to all optical spectroscopic instruments. These instruments generally have a light source, a wavelength selector, a sample holder, and a detector.

A. The Electromagnetic Spectrum. $c = \lambda\nu$, wavenumber $[\lambda] = 1/\lambda$.

Spectral domains (memorize): UV: 190 $\leftrightarrow$ 400 nm, VIS: 400 $\leftrightarrow$ 700 nm, near IR: 700 nm $\leftrightarrow$ 2500 nm (2.5 μm), IR: 2.5 μm $\leftrightarrow$ 25 μm (4000 $\leftrightarrow$ 400 cm^{-1}), far IR: $> 25 \mu\text{m}$ ($< 400 \text{ cm}^{-1}$). Note characteristic units of wavelength and wavenumber.

B. Refraction and Reflection.

The index of refraction n is inversely proportional to the velocity of electromagnetic radiation through a substance. It is 1 by definition through vacuum, and very nearly 1 in air. The index of refraction of a substance depends on the identity of the substance and on optical dispersion (for a transparent substances, the index of refraction increases with decreasing wavelength). This has important consequences for lenses and prisms.

When light passes through an interface between two substances with different indices of refraction, it undergoes refraction and reflection. The direction of the refracted light changes according to Snell's law:

$$(\sin \theta_1)/(\sin \theta_2) = n_2/n_1$$

where θ_1 and θ_2 are the angles of incidence and angle of refraction, respectively, and n_1 and n_2 are the indices of refraction of the two substances. This formula can be used to calculate the path of light through lenses and prisms.

Some of the light is reflected. Let R = reflected intensity fraction, T = transmitted intensity fraction, so that $T + R = 1$ (both quantities are often expressed as percentages). At 0° angle of incidence (light beam perpendicular to the interface):

$$R_{12} = [(n_2 - n_1)/(n_2 + n_1)]^2$$

A large mismatch in indices of refraction causes significant reflection losses.

Ex. Infrared light passes from air into a NaCl crystal. n for NaCl at IR wavelengths is 1.50. Calculate R_{12} and T_{12} .

$$R_{12} = [(1.50 - 1.00)/(1.50 + 1.00)]^2 = 0.040; \quad T_{12} = 1 - R_{12} = 0.960$$

4.0% of the light is reflected, 96.0% is transmitted.

For multiple interfaces, $T_t = T_1 \cdot T_2 \dots$. Note: the SH&N text states that you add the R values. This approximation is only correct for small R values.

Ex. A quartz cuvette holding an aqueous solution has 4 interfaces: air/quartz, quartz/water, water/quartz, quartz/air. Calculate the fraction of transmitted light passing through all 4 interfaces. n_1 (air) = 1.00, n_2 (quartz) = 1.47, n_3 (water) = 1.33.

$$R_{12} = [(1.47 - 1.00)/(1.47 + 1.00)]^2 = 0.036; \quad R_{23} = [(1.33 - 1.47)/(1.33 + 1.47)]^2 = 0.0025$$

For this formula, reflection loss is independent of the direction of the light, so $R_{32} = R_{23}$ and $R_{21} = R_{12}$. Hence: $T_{12} = 0.964 = T_{21}$; $T_{23} = 0.9975 = T_{32}$; $T = (0.964)(0.9975)(0.9975)(0.964) = 0.925$ or 92.5%

C. Optical Materials for Windows, Cuvettes, Lenses, Prisms.

Reading:

SH&N, ch. 7, pp. 143-154, 167-178, 154-166 (order in which topics will be covered)

Memorize this list of common optical materials!

UV: Quartz or silica (SiO_2) (most common).
 VIS & near IR: Glass or quartz. (note: glass (e.g., Pyrex) absorbs strongly below 330 nm)
 IR: Br^- , Cl^- or F^- salts of Na^+ or K^+ (all water-sensitive), CaF_2 , Ge, Si, AgCl.

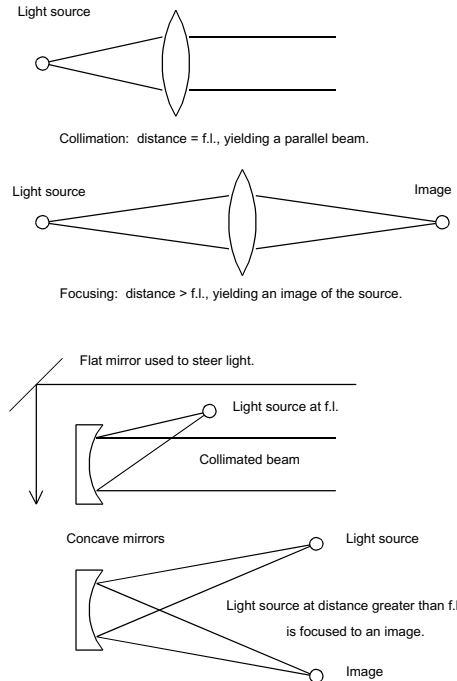
D. Lenses and Mirrors. (note: this material is not in SH&N. It is a review of basic optics.)

Lenses are characterized by their focal length (f.l.) and f# (ratio of f.l. to aperture diameter). Lenses with lower f# collect more light but have greater aberrations (deviations from ideal behavior). Two basic functions of lenses: collimation (produce parallel beam of light) and image formation (focus light to an image of the source).

Mirrors surfaces are highly reflective ($R \rightarrow 1$). Like lenses, mirrors are characterized by their f.l. and f#. Three functions: steering (to change the direction of the beam), collimation and image formation. Mirrors are now used predominately in the UV/VIS and almost exclusively in the IR because (a) metals have R values very close to 1, and (b) reflection losses for lenses.

Both lenses and mirrors suffer from aberrations, which cause stray light (light rays that travel undesired paths).

Review



Definitions: Wavelengths and/or wavenumbers for the UV, VIS, near IR, mid-IR and far IR regions; index of refraction; optical dispersion; collimation; focal length and $f\#$ for convex lenses and concave mirrors.

Concepts: What optical materials are suitable for use in the UV, VIS + near IR, or IR regions?

Calculations: Snell's law; reflection losses for one or more interfaces given the indices of refraction.

Most optical spectrometers have the following common components: light source, sample holder, wavelength selector (usually a monochromator), light detector, and signal processor. The following sections survey the choices for light source, light detector, and wavelength selector.

Reading: Harris, ch. 20, pp. 463-476.

VI. LIGHT SOURCES.

- A. Continuum Sources - light emission over a broad range of wavelengths; reasonably constant intensity with time.
1. Black body emitters - hot filaments or rods of metal (tungsten) or ceramics. Characteristic emission spectrum of a black body emitter: sharp cutoff on short wavelength side; long tail on long wavelength side. VIS and near IR sources are encased in glass or quartz; IR sources operate naked. Not suitable for UV.
 2. High pressure arc lamps - DC current arc through high pressure gases of Xe, Hg or a mixture of both. Xe arc resembles sunlight. Very bright emission covers UV, VIS, and near IR; wavelengths associated with Hg or Xe atomic emission lines are much more intense. Eye hazard. More expensive and less stable than W filaments; ozone (toxic hazard) is formed around the lamp by the intense UV radiation. A related source is the Xe flash lamp. A current pulse through two electrodes in argon produces a high intensity and very short (sub-microsecond) white light flash.
 3. D₂ arc lamp - DC arc through D₂ at low pressure produces light in the UV and VIS. Used as UV and VIS source, but not near IR.

B. Line sources.

Light emission at a few discrete wavelengths; generally constant intensity with time. A special line source, the hollow cathode lamp, is discussed in atomic absorption spectroscopy.

C. Lasers.

Based on stimulated emission. A photon striking an excited atom causes a second photon to be emitted at precisely the same wavelength and phase. Lasers are line sources. They may be continuous wave (CW) or pulsed. Advantage: extremely intense source of monochromatic light (one or a few wavelengths). Dye lasers are tunable over a narrow range (40 - 80 nm width). Common lasers include HeNe (632.8 nm, CW), Ar ion (488.0, 514.5 & 568.2 nm, CW), CO₂ (ca. 10,000 nm or 1000 cm⁻¹, CW), N₂ (337 nm, pulsed), Nd:YAG (1030 nm, pulsed), and semiconductor diode lasers (near IR and red visible wavelengths, CW or pulsed). In general, their lack of tunability over a wide wavelength range limits the use of these sources in optical spectroscopy.

Definitions: continuum and line sources.

Concepts: What are the 3 common continuum sources? Over what spectral regions are they useful?

VII. DETECTORS.

Detectors convert light (UV, VIS, IR) into an electrical signal (usually current or voltage). There are many types of detectors. The discussion will focus on the most important ones.

The main detector parameters of interest are the relative sensitivity and the operating wavelength range.

A. Quantum detectors (photon in, electron out).

1. Vacuum phototube - based on photoelectric effect. Photon kicks out electron from photocathode covered with low work function metal or alloy (sketch). The photoelectron is collected by the anode which is 80 - 100 V positive of cathode. Output is current. Cheap, moderately sensitive, useful in the UV-VIS. Vacuum phototubes are blind to IR light because photons don't have enough energy to kick an electron out of any photocathode.
2. Photomultiplier (PMT) - photocathode plus dynodes plus anode (sketch). Photoelectron from photocathode hits 1st dynode (100 V positive of photocathode), kicking out 3 - 6 more electrons, which hits 2nd dynode (100 V positive of 1st cathode), each kicking out 3 - 6 more electrons, etc. Overall internal gains of 10^5 to 10^8 are common for 8 - 14 dynodes. Very sensitive (single photon detection possible), very wide usable range of intensities measureable (3 to 6 orders of magnitude), hence very widely used. Useful in the UV-VIS.
3. Photodiodes - light absorbed by semiconductor diode creates hole-electron pair. Signal output is either the current flow, or change in conductivity. Cheap, sensitive, spectral range determined by semiconductor (for UV-VIS, Si; for near IR, PbS; for IR, Ge or HgCdTe (mercury cadmium telluride, or MCT)). IR detectors require cooling to LN_2 or liquid He temperatures. Readily fabricated into multichannel detectors (linear photodiode arrays).
4. Charge-transfer detectors (CTD's) - Very sensitive and expensive detectors based on semiconducting silicon which can measure intensity versus position in a 2-dimensional image. Includes charge-coupled devices (CCD's). Useful in the UV-VIS. Starting to replace PMT's.

B. Thermal detectors - light converted to heat; temperature change detected. Consider only the

Pyroelectric detector - based on pyroelectric crystal used as dielectric in a capacitor. Temperature change of crystal changes the capacitance, inducing a transient current. Works best when light is modulated at frequencies of 500 to 5000 Hz. Sensitivity is poor; used mainly in Fourier Transform IR spectrometers. The most important pyroelectric crystal is deuterated triglycine sulfate (DTGS).

Review

Definitions: quantum and thermal detectors; MCT, CTD, CCD, DTGS.

Concepts: What are the 4 types of quantum detectors? How does a PMT work (include a sketch)? Why are vacuum phototubes and PMTs blind to IR wavelengths?

The next two sections focus on wavelength selectors.

VIII. OPTICAL FILTERS.

Function - to remove unwanted wavelengths, or to make a nearly monochromatic beam from a continuum source. Two types: bandpass or cutoff.

1. Cutoff filters strongly absorb light below (low pass) or above (high pass) a specified wavelength. Sketch of transmittance spectra. Based on natural light absorption of substances.
2. Bandpass filters transmit light over a narrow range of wavelengths. They are characterized by peak wavelength (λ), peak transmittance (T as defined on p. 8), and bandpass ($\Delta\lambda$ = range of wavelengths at half-peak transmittance). Interference filters transmit a narrow range of wavelengths based on constructive/destructive interference for light bouncing between two reflectors separated by a dielectric layer.

$$\lambda = 2dn/m$$

where d is the thickness of the dielectric, n is the index of refraction of the dielectric layer, and m is the order of the wavelength (1st order, m = 1; 2nd order, m = 2, etc.). typical bandpasses are 1-10 nm. Available for wavelengths in the UV-VIS-near IR domains. Usually low pass cutoff filters are used to block the higher order wavelengths in monochromators.

Ex. An interference filter is constructed using a dielectric layer with a thickness of 214 nm and an index of refraction of 1.40. What are the 1st, 2nd, and 3rd order wavelengths transmitted by the filter? How could a cutoff filter be used with the interference filter to limit the transmitted light to just the first order wavelength?

For first order wavelengths, $\lambda = 2(214 \text{ nm})(1.40)/1$; $\lambda = 600 \text{ nm}$. The filter will transmit 2nd order light at 300 nm, 3rd order light at 200 nm, etc. Combine the interference filter with a low pass cutoff filter to block wavelengths shorter than 500 nm, and only the 600 nm light will be transmitted.

Review

Definitions: cutoff, bandpass & interference filters.

Calculations: the wavelengths associated with each order in an interference filter, given the thickness and index of refraction of the dielectric layer.

IX. MONOCHROMATORS.

The key function of a monochromator is to transmit light of a selected wavelength, which can be scanned manually or automatically. Input may be a continuum source ("white" light) or a mixture of monochromatic wavelengths (line source). The following discussion will be limited to monochromators using mirrors and diffraction gratings.

A. Basic Elements.

1. Entrance slit - provides a light source of well-defined dimensions (usually rectangular with width W & height h).
2. Collimator - a mirror which produces a collimated beam. Defined by f.l. and f#.
3. Diffraction grating - separates different wavelengths by their angle of diffraction. Defined by angular dispersion (see below).
4. Focusor - a mirror which produces an image of the entrance slit on the focal plane. Usually same f.l. and f# as collimator.
5. Exit slit - isolates the entrance slit image for a narrow range of wavelengths. Located at the focal plane of the focusor.

Ex. a Czerny-Turner monochromator. Light from an external light source is focused through the entrance slit. The light beam is collimated by the collimator and directed at the diffraction grating. Light of different wavelengths is diffracted at different angles. The collimated diffracted beam is focused to a series of images of the entrance slit along the focal plane, each image having a characteristic wavelength. The exit slit only allows a narrow range of wavelengths to exit the monochromator. Wavelength scanning is achieved by rotating the grating, which moves the images of the entrance slit along the focal plane.

B. Monochromator Parameters.

The performance of the monochromator is very important in determining the overall performance of any spectrometer. Selected performance parameters are (units are selected for the UV/VIS region):

1. Bandwidth ($\Delta\lambda$): Refers to the range of wavelengths passing through the exit slit.
2. Resolution (a.k.a. resolving power) (R): $\lambda/\Delta\lambda$ (unitless). Refers to the ability of the monochromator to distinguish between two closely spaced wavelengths; λ is the average wavelength. Ex. Minimum resolution required for resolving sodium D lines at 588.996 and 589.593 nm is:

$$R = \frac{1}{2}(589.593 + 588.996)/(589.593 - 588.996) = 987$$
3. Throughput: ratio of output intensity to input intensity (same as transmittance).
4. Focal length and f#: Refers to the focal length and f# of the collimator and focusor (usually the same). Focal length (f.l.) defines the size of the monochromator; throughput is inversely proportional to f#.
5. Stray light: light of wrong wavelengths (i.e., wavelengths outside the bandwidth) passing out the exit slit.

The following two parameters will not be covered in lecture. They will appear in the homework, but not on exams.

6. Angular dispersion (A.D.): $dr/d\lambda$ in radians/nm. Refers to the difference in angle of two closely spaced wavelengths (units nm) leaving the grating (see below).

7. Linear dispersion (D): $dy/d\lambda$ in mm/nm. Refers to the spread of different wavelengths along the focal plane. Often shown as the linear reciprocal dispersion $D^{-1} = d\lambda/dy$ in nm/mm. Be careful with units! The units of D are mm of distance along the focal plane per nm of wavelength.

C. Diffraction Gratings.

A reflection diffraction grating (the most common kind) consists of narrow parallel mirrors on a planar surface. The mirrors break a collimated beam into N evenly spaced rectangular sources. Rays from all the sources constructively interfere at particular angles and wavelengths. The mirrors are separated by grating spacing d, which is roughly the same length as the wavelength of the diffracted light; hence different gratings are used in different spectral regions. Gratings are also characterized by $1/d = \# \text{ blazes/mm}$, and $w = \text{width of grating}$ ($w = N \cdot d$).

Grating equation: $\lambda = (d/m)(\sin i + \sin r)$ (The + sign means that the incident and diffracted beam are on the same side with respect to the surface normal. In Harris, a - sign is used because the incident and diffracted beam are on the opposite sides of the surface normal.)

where i = angle of incidence; r = angle of diffraction; m = order = 1, 2, 3, ... The 1st order wavelength is the longest; higher order wavelengths are integer fractions of the 1st order wavelength. Usually the higher order beams are removed by inserting a low pass cutoff filter in the exit beam; the cutoff filter has to be changed as the monochromator scans wavelengths.

Ex. What wavelengths (in nm) are diffracted at an angle of 20° if a collimated beam of white light strikes a 1200 blazes/mm grating at an angle of 30°?

$$d = 1/(1200 \text{ blazes/mm}) = 8.33 \times 10^{-4} \text{ mm}(10^{-3} \text{ m/1 mm})(1 \text{ nm}/10^{-9} \text{ m}) = 833 \text{ nm}$$

$$\lambda = (833/m)(\sin 30^\circ + \sin 20^\circ) = (701 \text{ nm})/m = 701 \text{ nm} (m = 1), 351 \text{ nm} (m = 2), \dots$$

A low pass cutoff filter which blocks light below 500 nm would block all of the higher order wavelengths.

Types of gratings:

1. Echellette or blazed gratings have sawtooth surfaces; the broad side of the sawtooth is used. They are characterized by a blaze wavelength (λ_b). A blazed grating has high throughput from $2\lambda_b$ to $(2/3)\lambda_b$; hence a blaze wavelength of 300 nm is good for the UV/VIS (200-600 nm). Most gratings today are blazed.
2. Echelle gratings use the short side of the sawtooth surface; they are used at high orders ($m > 10$) with i and r above 60°. These gratings yield very high resolution. Order sorting is required (this will be discussed in the section on atomic emission spectrometers)
2. Concave gratings diffract light and produce an image of the entrance slit; hence the collimator and focusor are eliminated in the monochromator. Chief advantage: higher throughput.

For all gratings, the theoretical maximum resolution $R = m \cdot N$; hence for a fixed grating spacing, wider gratings have better resolution, and echelle gratings (high m) have extremely high resolution.

The following section will not be covered in lecture, nor will it appear on exams. It will be needed for a homework set.

D. Calculations of grating monochromator performance parameters.

For fixed angle i :

$$A.D. = dr/d\lambda = m/(d \cdot \cos r) \approx m/d \quad (\text{assumption: } r \text{ is small})$$

Gratings used to pass 1st order wavelengths operate at relatively low values of r , so $\cos r$ is nearly 1.

The other factors affecting monochromator performance are the focal length, the $f\#$, and the slit widths.

The linear dispersion is simply the product of the A.D. and the focal length of the focuser:

$$D = (A.D.)(f.l.); \quad D^{-1} = 1/[(A.D.)(f.l.)]$$

Again, if r is small, then $\cos r \approx 1$, and the linear dispersion is nearly independent of the wavelength.

Ex. A 2000 blazes/mm grating is placed in a monochromator with a 0.25 m f.l. Calculate A.D., D , and D^{-1} for the 1st order wavelengths.

$$d = 1/(2000 \text{ blazes/mm}) = 5.0 \times 10^{-4} \text{ mm} (10^6 \text{ nm/mm}) = 500 \text{ nm}$$

$$A.D. \approx m/d = 1/500 \text{ nm} = 2 \times 10^{-3} \text{ radians/nm}$$

$$f.l. = (0.25 \text{ m})(1000 \text{ mm/m}) = 250 \text{ mm}$$

$$D = (250 \text{ mm})(2 \times 10^{-3} \text{ radians/nm}) = 0.5 \text{ mm/nm}; \quad D^{-1} = 1/(0.5 \text{ mm/nm}) = 2.0 \text{ nm/mm}$$

The bandwidth of the light exiting the monochromator is simply the product of the slitwidth and the linear reciprocal dispersion:

$$\Delta\lambda = (W)(D^{-1}) \quad (\text{note: the units of slitwidth and distance along the focal plane must match})$$

Ex. What is D , D^{-1} , $\Delta\lambda$ and R at 500 nm if $A.D. = 8 \times 10^{-4} \text{ rad/nm}$, $W = 0.10 \text{ mm}$, and $f.l. = 0.15 \text{ m}$?

$$D = (8 \times 10^{-4} \text{ rad/nm})(150 \text{ mm}) = 0.12 \text{ mm/nm}; \quad D^{-1} = 8.3 \text{ nm/mm}$$

$$\Delta\lambda = (0.10 \text{ mm})(8.3 \text{ nm/mm}) = 0.83 \text{ nm}$$

$$R = (500 \text{ nm})/(0.83 \text{ nm}) = 600$$

The more expensive monochromators have slits of adjustable width W . For a continuum input (“white” light), throughput is proportional to slit area $\times$ bandpass $\approx W^2$. In other words, decreasing the slitwidth by a factor of 2 decreases the throughput by a factor of 4.

Summary of factors affecting monochromator performance:

1. The grating must be matched to the spectral region. The grating spacing d must be similar to the wavelengths of light being passed by the monochromator. The blaze wavelength of the grating should fall in the middle of the operating wavelength range.
2. Grating monochromators pass wavelengths of all orders. A low pass cutoff filter must be inserted in the beam if only the 1st order wavelength is desired. The filter must be changed periodically as the monochromator wavelength is scanned.
3. Four factors affect bandwidth and resolution: the # blazes/mm (i.e., the grating spacing), the order of the wavelength, the f.l. of the monochromator, and the slit width. A grating monochromator with a small bandwidth (and higher resolution) has a grating with a high # blazes/mm (small d spacing), a large f.l., and a small slit width. Certain specialized monochromators operate at higher order wavelengths.
4. Three factors affect throughput: the blaze wavelength of the grating, the $f\#$ of the collimator and focuser, and the slit width. To get higher throughput, choose a grating whose blaze wavelength is close to the desired wavelengths, choose a monochromator with a smaller $f\#$, and increase the slit width.

E. Stray light.

Sources: (a) imperfections in optical elements; (b) reflections from lenses; (c) wrong orders; (d) diffraction from slits; (e) dust in the air. Stray light is especially a problem near the extreme wavelength settings of the monochromator. Stray light can be reduced by using (1) internal baffles, (2) flat black internal surfaces, (3) sealed optics. Very low levels of stray light are achieved with a double monochromator - the exit slit of the 1st monochromator is the entrance slit of the 2nd monochromator. Both monochromators are tuned to the same wavelength. Triple monochromators have extremely low levels of stray light, but are very expensive.

Review

Definitions: monochromator; angular dispersion; bandwidth; resolution; throughput; focal length and $f\#$; echellette and echelle gratings; blaze wavelength; stray light.

Concepts: What are the 5 basic elements of a monochromator? Sketch the Czerny-Turner monochromator with either a planar or a concave grating. How is the wavelength scanned? How is stray light reduced? How is a grating matched to a spectral range? Why must cutoff filters be used with grating monochromators?

Calculations: Calculate the minimum Resolution needed to resolve two spectral lines. Calculate the wavelength of a given order from the grating equation. Calculate the maximum theoretical resolution of a grating.

Overview: The following topic applies to spectrometric instruments that measure absorbance: UV-VIS and IR spectrometers, atomic absorption spectrometers, UV-VIS detectors for liquid chromatography.

X. QUANTITATIVE AND QUALITATIVE ANALYSIS BY SPECTROSCOPY.

Reading: Harris, ch. 18, pp. 407-420, ch. 19, pp. 434-437

SH&N, ch. 6, pp. 139-140; ch. 13, pp. 300-306, 311-312; ch. 14, pp. 342-345.

A. Energy State Diagrams

All of the spectroscopic methods to be discussed are based on the interaction of light (photons) with the analyte atom or molecule. The fundamental interactions are:

Absorption: a photon is absorbed, and the atom or molecule becomes excited.

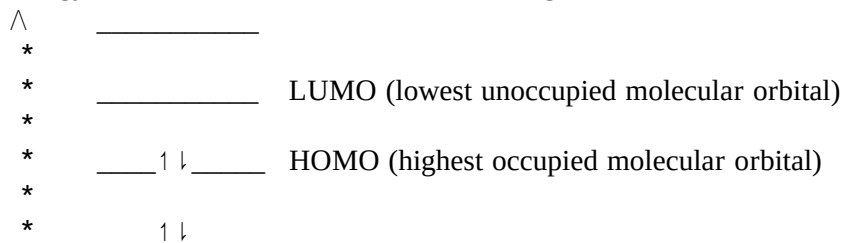
Emission: a photon is emitted, and the atom or molecule is no longer excited (in its ground state)

Scattering: a photon is absorbed and emitted at virtually the same instant

To better understand what each type of instrument is measuring, it is helpful to discuss the three interactions in terms of an energy state diagram. The following discussion is limited to light absorption by molecules. Other cases will be discussed in the relevant sections on other types of spectroscopy (fluorescence, Raman, atomic absorption, atomic emission).

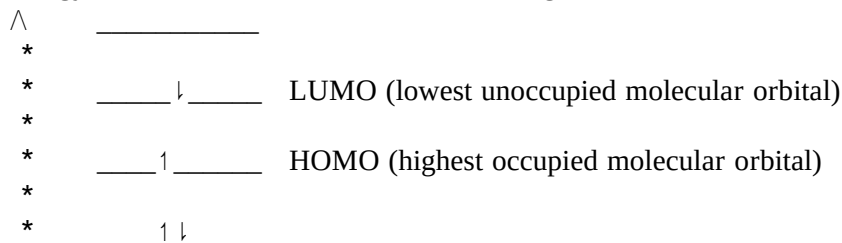
In previous chemistry courses, you were probably introduced to a Molecular Orbital (MO) diagram. The energies of orbitals are represented by a set of horizontal lines, with energy increasing vertically. When a molecule is in its ground state (having the lowest amount of electronic energy possible), then electrons occupy the lowest available MO's (diagram - electrons are filled up to the HOMO). In most molecules, there are an even number of electrons, and the spins of all the electrons are paired. The molecule is said to be in a singlet state because a magnetic field does not cause any splitting of the energy state. The ground singlet state of a molecule is labeled S_0 .

Energy Molecular orbital diagram of a molecule in the S_0 state.



If a molecule absorbs a photon of light of sufficient energy, then an electron is promoted to a higher energy MO. Since the electron spin does not change during absorption of a photon, the molecule is still in a singlet state, but now it is in an excited state. The lowest excited state is designated S_1 ; an electron resides in the LUMO. Higher excited states are S_2 , S_3 , and so on.

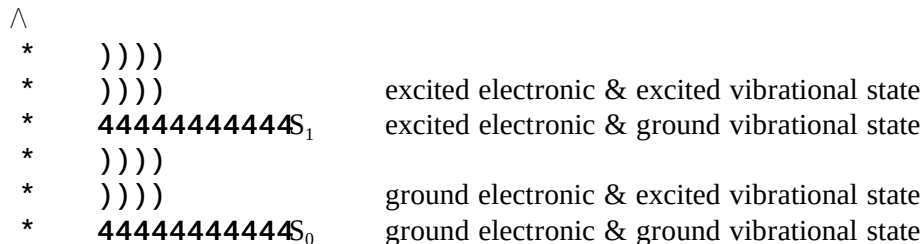
Energy Molecular orbital diagram of a molecule in the S_1 state.



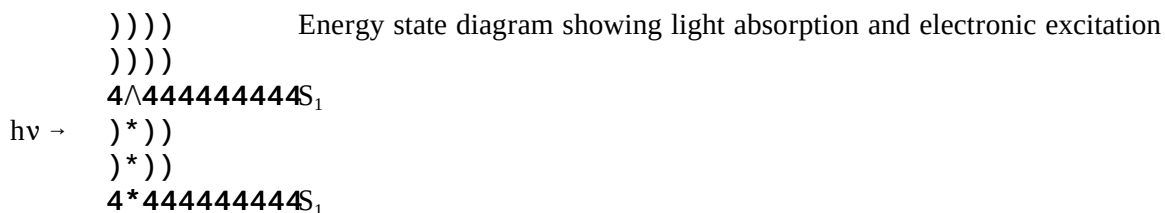
An energy state diagram represents each energy state as a horizontal line. Energy is increasing vertically. Electronic states (S_0 , S_1 , etc.) are represented with long double lines. The distance between the double lines is the energy needed to change the molecule from one energy state to another.

By the same argument as above, there are ground vibrational states (molecule showing the least amount of vibration) and excited vibration states (molecule vibrating more vigorously). These are usually shown as short light lines. The energy needed to raise a molecule to an excited vibrational spacing between vibrational states is much smaller than the energy needed to raise the molecule to an excited electronic state, so the spacing is smaller.

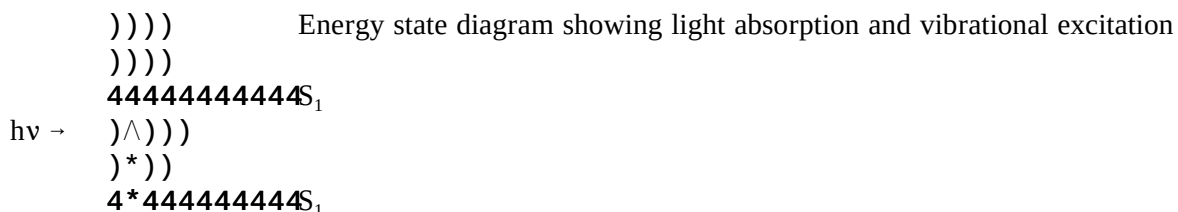
Energy Energy state diagram of a molecule showing electronic & vibrational states



At room temperature, almost all molecules are in the ground electronic & ground vibrational state. If a photon of light has energy ($E = h\nu$, where ν is the frequency of light and h is Planck's constant) equal or greater than the distance between S_0 and S_1 in the energy state diagram, then it is possible for the photon to excite the molecule electronically. For most molecules, this requires light in the UV and possibly the VIS spectral range.



If the photon of light has energy equal or greater than the distance to the vibrational excited states, then the photon can excite the molecule vibrationally. For most molecules, this requires light in the IR spectral range.



An absorption spectrum is a plot of the probability of the photon being absorbed as a function of its energy (often expressed in other units, such as wavelength for UV-VIS spectroscopy and wavenumber for IR spectroscopy). Absorption spectroscopy can provide qualitative analysis through the absorption spectrum. The magnitude of absorption (see section B) at a given photon energy (wavelength) yields concentration (quantitative analysis). In practice, UV-VIS absorption spectroscopy is mainly used for quantitative analysis and IR absorption spectroscopy is mainly used for qualitative analysis.

B. Beer's Law.

Beer's Law is the most widely applied method for determining concentrations by spectroscopy. This Law is valid in any spectral domain for any spectral transition if the assumptions are valid; it is most commonly applied in the UV/VIS.

Assumptions:

1. The analyte absorbance is constant over the bandwidth $\Delta\lambda$ of the transmitted beam.
2. The analyte molecules (ions, atoms) do not interact (infinite dilution approximation, usually valid).
3. The concentration is uniform across the sample.

Define absorbance A (unitless) as: $A = -\log(T) = \log(1/T) = \log(P_0/P)$

where P_0 is the incident power, P is the transmitted power, and T is expressed as a decimal fraction.

Then: $A = \epsilon bC$ (Beer's Law)

where ϵ = molar absorptivity (units $M^{-1}cm^{-1}$); b = sample thickness (cell pathlength) in cm; and C = conc. in M (mol/L). ϵ is a property of the analyte and of wavelength; identification of the analyte (qualitative analysis) is possible from the spectrum (ϵ vs λ). Note that the sensitivity m is equal to ϵb .

Be aware of how absorbance and transmittance scale with respect to each other: $T = 10^{-A}$

<u>T</u>	1.	0.1	0.01	0.001
<u>%T</u>	100%	10%	1%	0.1%
<u>A</u>	0.0	1.0	2.0	3.0

For absorbances above 2, less than 1% of the incident light is exiting the sample. This has significant consequences in absorption spectrometers; accuracy decreases and noise increases in absorption measurements.

Ex. The molar absorptivity of MnO_4^- is $2.3 \times 10^3 (M \cdot cm)^{-1}$ at 520 nm. What conc. of permanganate would yield an absorbance of 0.80? What is the transmittance of this solution? If the permanganate solution is diluted to $\frac{1}{2}$ of its initial conc., what is the transmittance of the diluted solution? Assume b = 1.0 cm.

$$0.80 = (2.3 \times 10^3 (M \cdot cm)^{-1})(1.0 \text{ cm})C; \quad C = 3.5 \times 10^{-4} \text{ M}$$

$$T = 10^{-0.80} = 0.16 \text{ or } 16\%$$

Since A is proportional to C, dilution to $\frac{1}{2}$ of the initial conc. decreases A by a factor of 2.

$$A = 0.80/2 = 0.40; \quad T = 10^{-0.40} = 0.40 \text{ or } 40\% \text{ (note that T is NOT doubled)}$$

To maximize sensitivity, one normally chooses a wavelength at which ϵ exhibits a peak. For solid or solution samples, the pathlength is normally 0.1 to 1.0 cm, but for gas samples, very long pathlengths can be used.

A consequence of assumption (2) is that absorbances of separate analytes are additive:

$$A_t = \Sigma A_i = b \Sigma \epsilon_i C_i \text{ at any given wavelength.}$$

A sample containing 2 analytes can be analyzed by measuring absorbance at 2 wavelengths. One can then set up two equations in two unknowns and solve for both concentrations.

$$A_1 = b(\epsilon_{x1}C_x + \epsilon_{y1}C_y) \text{ (at } \lambda_1\text{)}$$

$$A_2 = b(\epsilon_{x2}C_x + \epsilon_{y2}C_y) \text{ (at } \lambda_2\text{)}$$

where X and Y are the two analytes. It is necessary to know the 4 molar absorptivities of each compound at the 2 wavelengths. For greatest accuracy, λ_1 and λ_2 are chosen so that the ratios $\epsilon_{x1}/\epsilon_{y1}$ and $\epsilon_{x2}/\epsilon_{y2}$ are very different.

C. Errors in Quantification.

Calibration curves are called Beer's law plots (A vs C). The sensitivity is $\epsilon \cdot b$; the limit of detection is set by absorbance noise of blank samples. A Beer's law plot is the best method for determining ϵ . Systematic errors (general & instrumental) cause positive or negative deviations in Beer's law plots.

1. General.

Chemical equilibria - the analyte exists in a chemical equilibrium of 2 or more forms which shifts with changing concentration. Includes dimerization, acid-base, and complexation equilibria. A clear indication of an equilibrium between two forms (e.g., the acid and base forms of an indicator) is obtained by creating an overlay plot of ϵ vs λ at different equilibria conditions (e.g., different solution pH)(sketch). The different curves will intersect at a wavelength at which the molar absorptivities of the two forms are equal. This intersection point is called an isosbestic point.

2. Instrumental.

- (a) Absorbance not constant over bandwidth wavelengths - either the bandwidth $\Delta\lambda$ of the spectrometer is too large relative to the peak width, or the wavelength selected is on a steeply changing region of ϵ . The apparent absorbance is less than the true absorbance. A Beer's law plot will exhibit a negative deviation. Always choose a peak or a valley wavelength for a Beer's law plot. This is a serious problem when absorption bands are narrow (absorption spectroscopy of atoms or molecules in the gas phase in all spectral regions, absorption spectroscopy of molecules in condensed phases in the IR region).
- (b) Stray light - light which is not absorbed by the sample reaches the detector, which causes negative deviation of the Beer's law plot. This is quite common for absorbances greater than 2, and even worse when the instrument is operating near its wavelength extremes.

An analysis of noise in an absorption spectrometer indicates that the best precision can be obtained for $A = 0.1$ to 1.0 ; in that range, relative errors less than 0.5% are possible (i.e., 3 sig. fig. accuracy).

D. Qualitative Analysis.

A plot of A (or ϵ) vs λ is called an absorption spectrum. Peak positions and relative peak heights are used to identify the analyte. To get an accurate spectrum, the bandwidth of the spectrometer should be no greater than 1/10th of the half-width of the narrowest absorption peak. This is especially a problem in IR spectroscopy. If the bandwidth is too large, smaller peaks can disappear, and relative peak heights are

distorted. Apparent absorption peak heights are smaller and apparent peak widths are larger than the true peak heights and widths. (see p. 470 of Harris)

If the spectrometer has adjustable bandwidth, it is wise to obtain spectra at two bandwidths. If they match well in peak heights and peak widths, then bandwidth is not a problem. If they don't, continue to decrease the bandwidth until no change is observed in the spectra.

Review

Definitions: Beer's law plot; isosbestic point.

Concepts: Sketch an energy state diagram showing absorption of light to form an electronic or a vibrational excited state. What assumptions must be true for Beer's law to be valid? What are the factors (both general and instrumental) that cause deviations from linearity in a Beer's law plot? How is an absorption spectrum distorted if the bandwidth of the monochromator is too large?

Calculations: Conversion between T, %T and A; Beer's law calculations for one or two analytes; sensitivity from a Beer's law plot.

XI. UV/VIS/near IR SPECTROMETERS.

Reading: Harris, ch. 20, pp. 461-463.

SH&N, ch. 13, pp. 312-325. Reading of ch. 14, pp. 329-342, is highly recommended.

A. General Design Principles.

Basic block diagram of an absorption spectrometer. The wavelength selector and sample can be interchanged.

Source → Wavelength Selector → Sample → Detector → Readout

Quick review of the options:

5. Source - continuum. In order of increasing cost: tungsten filament (VIS-near IR), D₂ lamp (UV-VIS), and high pressure Xe arc (UV-VIS-near IR).
6. Wavelength Selector - optical filter or grating monochromator. The monochromator wavelength can be manually set or automatically scanned.
7. Sample - For solutions, a cuvette made of glass (Pyrex), clear plastic, or quartz (fused silica). Glass and plastic are OK for the VIS-near IR, quartz is necessary for the UV and is usable in the VIS and near IR.
8. Detector - UV-VIS: In order of increasing cost: silicon photodiode, vacuum phototube, photomultiplier, linear diode array, CCD. Near IR: PbS photodiode.

The objective is to measure the transmittance of the sample. All spectrometers need to make 3 measurements to obtain the transmittance:

- (a) Dark current - the light beam is blocked and the dark current flowing through the detector is measured for later, or the readout is adjusted to read 0%T.

- (b) Incident light power P_0 .
- (c) Transmitted light power P .

The last 2 parameters must be measured every time the wavelength is changed.. Because of reflection losses in the cuvette (about 6-8%; see p. 8 of the Required Notes), P_0 is usually measured using a blank i.e. a cuvette filled with just solvent.

B. Spectrophotometers (includes a monochromator).

1. Single beam.

The operating wavelength range is determined by the source, detector and monochromator; the wavelength and bandwidth are set by the monochromator.

Ex. B&L Spectronic 20: W filament source, vacuum phototube detector, grating monochromator plus cutoff filter, display is a dial linear in transmittance. Probably the most widely used UV-VIS spectrometer in the world. Normal range 350 - 650 nm, extending to 950 nm with a red-sensitive phototube; bandpass 20 nm. A second phototube directly monitors the source intensity to correct for source flicker (not a true double-beam instrument). Stray light is a serious problem at wavelength extremes; the large bandpass distorts absorption spectra. Useful for Beer's law plots up to an absorbance of 1 (10% T). To correctly measure the transmittance, follow this procedure:

- (a) Block the light beam; adjust 0% T or dark current until the meter reads 0%.
- (b) Insert the blank sample (usually just the solvent) in the cuvette; adjust 100% T until the meter reads 100%.
- (c) Insert the sample in its cuvette; read %T and calculate A.

2. Double beam.

Measure P and P_0 (nearly) simultaneously. This allows the monochromator to scan automatically, and reduces the error in the measured transmittance. A common design uses a rotating sector mirror. Light passes alternately through the sample and the blank at frequency f_0 . Both beams are directed to one detector. After detection, the signal is amplified selectively at f_0 (synchronous detection). P and P_0 are converted to T or A . As a general rule, double beam instruments use a PMT detector, have both a UV (D_2 lamp) and a VIS (W lamp) source, and have a useful dynamic range of 0.1 to 2 absorbance units.

3. Multichannel.

The sample is placed between the source (W filament or D_2 lamp) and the monochromator. In the monochromator, a concave grating focuses the entire spectrum onto a linear diode array located at the focal plane. All wavelengths are detected simultaneously. The bandwidth is fixed by the linear reciprocal dispersion of the monochromator and the spacing of the diode array. Typical bandwidths are 2 nm per diode, which is usually adequate in the UV/VIS domain. Spectra are obtained very quickly (< 1 s). There are no moving parts, so the wavelength reproducibility is excellent. The design is inherently single beam, so a blank spectra must be acquired first and stored in computer memory. Stray light can be a problem near 200 nm (the lower wavelength extreme).

Review

Definitions: single and double beam spectrometers, multichannel spectrometers.

Concepts: Sketch a single beam, double beam or multichannel spectrometer. What determines the wavelength range of these instruments? What determines the bandpass? What are the common sources and detectors, and in which spectral domains are they used? What are the advantages of the double beam design? What are the advantages of the multichannel design?

XII. INFRARED SPECTROMETERS.

Reading: Harris, ch. 20, pp. 481-487.

SH&N, ch. 16, pp. 380-383, 389-392; ch. 7, pp. 182-189; ch.16, pp. 392-396. Read about IR absorption spectroscopy, then dispersive IR instruments first, then Fourier Transform spectroscopy, and finally FT-IR spectrometers. The lecture will focus only on mid-IR spectrometers. I recommend that you read ch. 17 (Applications of IR Spectrometry), but that material will not be covered.

B. IR Absorption Spectroscopy (instead of wavelength, we will use wavenumber ($\lambda^* = 1/\lambda$)).

Absorption of IR light by molecules are due to changes in vibrational and/or rotational states. Vibrational modes in a molecule include stretching and bending of a group of atoms. Selection rule: to observe a vibrational absorption band, the molecule dipole moment must change during the vibration. Ex. Symmetric stretching of linear CO₂ yields no absorption band, but anti-symmetric stretching yields a strong absorption band. The characteristic peak wavenumbers and relative intensities are useful for identifying groups of atoms in a molecule, so IR spectroscopy is mainly used for qualitative analysis.

Quantitative analysis is possible provided that the assumptions for Beer's law are obeyed. Molar absorptivities for strong IR bands are in the range of 10-500 M⁻¹cm⁻¹. Bandwidths ($\Delta\lambda^*$) of absorption bands due to vibrational transitions are relatively narrow (1-100 cm⁻¹) compared to the IR range (4000-400 cm⁻¹). Bandwidths of rotational transitions are much narrower; rotational absorption bands within a vibrational band are visible for gas phase analytes but are indistinguishable in condensed phase (solid or liquid) samples. For quantitative analysis, the effective bandwidth of the IR spectrometer must be 1 cm⁻¹ or less.

Almost all IR spectroscopy is done in open air. Two atmospheric gases exhibit IR absorption bands: water vapor (broad absorption bands with lots of rotational fine structure centered near 3700 cm⁻¹ and 1600 cm⁻¹) and carbon dioxide (intense double-lobed absorption band at 2350 cm⁻¹ and a sharp band at 660 cm⁻¹). These absorptions will appear in every IR spectrum unless a method is used to eliminate them.

B. Dispersive IR Spectrometer (uses a monochromator; virtually obsolete now). (NB: this material won't be covered in class, and you won't be responsible for it on tests).

Block diagram of a typical double beam design with synchronous detection:

Source → sample → chopper → monochromator → detector

The source is a black-body emitter(Nernst Glower or Globar); the detector is a thermal detector (not a pyroelectric detector). The optics and sample holders must be salts or semiconductors, many of which are sensitive to water vapor (e.g., KBr). Differences from UV/VIS instruments include:

1. The sample and blank are placed before the monochromator because (a) stray light from the source in the near IR is reduced and (b) radiation from hot samples is prevented from reaching the detector.

2. The double beam design is highly desirable to compensate for light absorption of H_2O and CO_2 in air.
 3. Older instruments only produced %T output due to null detection methods (the reference beam intensity is reduced mechanically until it matches the sample beam intensity). The tradition of viewing IR spectra as %T vs wavenumber continues to this day. My recommendation: use absorbance output whenever possible because peak shapes and relative intensities are not altered by changes in concentration.
 4. IR dispersive spectrometers usually change the grating around 2000 cm^{-1} to improve monochromator throughput since grating efficiencies are high only over a moderate range of wavelengths ($2/3\lambda_b$ to $2\lambda_b$). The horizontal scale increased by a factor of 2 as a result. Since most absorption bands occur in the $2000\text{-}400\text{ cm}^{-1}$ range, that display format is still customarily used.
- C. Fourier Transform IR Spectrometer (uses an interferometer).

Block diagram: source → interferometer → sample → detector

The source is a black body emitter (Nernst Globar); the detector is a DTGS pyroelectric detector at room temperature or a MCT quantum detector cooled with liquid nitrogen.

Basic principles: (1) The interferometer modulates each wavelength of light at a characteristic frequency in the audio range (100 - 5000 Hz). (2) The detector follows the modulation and yields a signal representing intensity vs time. (3) An ADC digitizes the signal. (4) A computer algorithm called the fast Fourier transform (FFT) converts the data to intensity vs frequency to obtain the absorption spectrum.

Major advantages over a dispersive IR instrument:

1. All wavelengths simultaneously pass through the sample and reach the detector (Fellgett's advantage, also known as the multiplex advantage). A complete spectrum is obtained in about a second; co-adding multiple scans greatly improves S/N. Commercial FTIR instruments can obtain an absorption spectrum on a monolayer of molecules on a flat metal surface (peak absorbance ≈ 0.001).
2. Throughput to the detector is large (Jacquinot's advantage); hence strongly absorbing or scattering samples can be analyzed, or S/N is improved.
3. The bandwidth ($\Delta\lambda^*$) (called resolution by SH&N) is constant over the entire spectrum.
4. Wavenumbers and hence peak positions are known very precisely ($\pm 0.02\text{ cm}^{-1}$).
5. Stray light is much less of a problem since it is not modulated at audio frequencies.

Operation: The Michelson interferometer receives collimated light from the source and splits it into 2 paths. One path length is fixed, the other varies continuously by scanning a moving mirror at constant velocity. On recombination at the beam splitter, each wavenumber undergoes periodic constructive and destructive interference. The intensity of the light is modulated sinusoidally at $f = 2 \cdot v \cdot \lambda^*$, where v is the mirror velocity in cm/s and λ^* is the wavenumber of the light. The detector output is sampled at regular time intervals (δt) by the ADC; the resulting P vs t data set is the interferogram. The computer uses the fast Fourier transform to convert the interferogram to a single beam spectrum (P vs λ^*). Once both the

sample and blank single beam spectra have been computed, transmittance or absorbance spectra are displayed or plotted. The bandwidth is set primarily by the distance the moving mirror travels while the interferogram is collected; a longer travel distance results in a smaller bandwidth.

Because the FTIR spectrometer is inherently single beam in design, peaks due to light absorption by H₂O and CO₂ vapor often appear in spectra (handout). They appear because the concentration of these vapors changes between the time the reference spectrum (no sample) is recorded and the time the sample spectrum is recorded. My advice: run a reference spectrum immediately before the sample spectrum. Some users purge the spectrometer with dry N₂ or dry CO₂-free air to reduce the problem.

Ex. A Michelson interferometer has a mirror velocity of 1.0 cm/s. What is the frequency of modulation of light of 2000 cm⁻¹?

$$f = 2v\lambda^* = (2)(1.0 \text{ cm/s})(2000 \text{ cm}^{-1}) = 4000 \text{ s}^{-1} = 4000 \text{ Hz}$$

Review

Definitions: Fellgett's advantage; Jacquinot's advantage; interferogram; fast Fourier transform.

Concepts: What is the selection rule for a vibrational mode to have an IR absorption band? What bandpass is needed for quantitative work in the IR? Sketch a FT-IR based on a Michelson interferometer and discuss how it obtains interferograms and converts them to spectra. Why are FT-IR's often purged with dry nitrogen? What are the advantages of an FT-IR over the dispersive instrument?

Calculations: Frequency of modulation in an FT-IR as a function of mirror velocity and wavenumber.

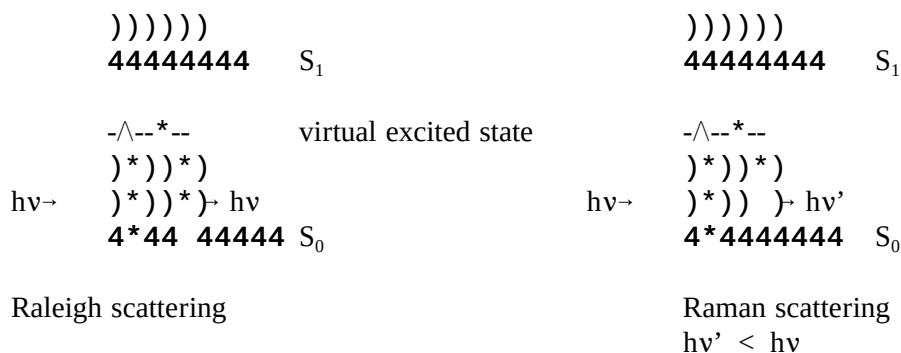
XIII. RAMAN SPECTROSCOPY.

Reading: Harris, p.179 (the box shows Raman spectra)
SH&N, ch. 18, pp. 429-432, 435-441.

Useful mainly for qualitative analysis, but uniquely suited for solids, surfaces, and aqueous solutions (e.g., biological samples, forensic analysis of paints & fibers, analysis of drugs in the powder form). Generates vibrational spectra while operating in the VIS or near IR domain. Optics and sample holders are usually glass or quartz.

A. The Raman Effect (as with IR, we will use wavenumber λ^*).

Based on light scattering by a (usually) transparent analyte (usually at wavelengths between vibrational and electronic transitions). An **energy state diagram** is useful. When a photon interacts with a molecule and has insufficient energy to raise the molecule to the lowest excited state, the molecule is raised to a virtual excite state for 10⁻¹⁴ s. Most of the time the molecule re-emits a photon at the same energy (wavelength) (Raleigh scattering). If the virtual state relaxes to a different vibrational state, then the re-emitted photon has a different energy (Raman scattering).



Raman scattering at smaller energy (longer wavelengths) yields Stoke's lines; anti-Stoke's lines appear at larger wavenumbers (shorter wavelengths). Both lines contain the same information (vibrational modes of the analyte), but Stoke's lines are usually more intense.

Ex. An Ar^+ laser excites a sample with 488.0 nm light. What wavelengths and wavenumbers correspond to Stoke's and anti-Stoke's lines of 3000 cm^{-1} ?

$$\lambda_{\text{ex}}^* = 1/\lambda = (1/488.0 \text{ nm})(1 \text{ nm}/10^{-9} \text{ m})(10^{-2} \text{ m/cm}) = (10^7 \text{ nm/cm})/(488.0 \text{ nm}) = 20492 \text{ cm}^{-1}$$

Stokes: $\lambda_s^* = 20492 \text{ cm}^{-1} - 3000 \text{ cm}^{-1} = 17492 \text{ cm}^{-1}$
 $\lambda_s = (10^7 \text{ nm/cm})/(17492 \text{ cm}^{-1}) = 571.7 \text{ nm}$

anti-Stokes: $\lambda_{\text{AS}}^* = 20492 \text{ cm}^{-1} + 3000 \text{ cm}^{-1} = 23492 \text{ cm}^{-1}$
 $\lambda_{\text{AS}} = 10^7/23492 = 425.7 \text{ nm}$

Thus, by measuring the energy (wavelength) of Raman scattered photons in the VIS range, information is obtained about the vibrational spectrum of the analyte substance. The normal range for vibrational spectroscopy ($100\text{--}5000 \text{ cm}^{-1}$) is compressed into a narrow wavelength range in the VIS ($< 100 \text{ nm}$).

Raman scattering efficiency is extremely small; roughly 1 photon in a million is Raman-shifted in wavelength. Usually the Stokes-shifted wavenumbers are observed.

The Raman spectrum is a plot of intensity vs wavenumber, usually from 50 to 4000 cm^{-1} (mid-IR and far IR information). Raman spectroscopy often complements IR absorption spectroscopy because the selection rules for allowed transitions are different. A Raman peak appears when a vibration mode causes a change in polarizability of bonds. The symmetric stretch of CO_2 is Raman-active.

B. Raman Instrumentation.

Like IR spectrometers, there are 2 types. Unlike IR spectrometers, they are of equal importance.

Block diagrams

Dispersive Raman spectrometer: source $\rightarrow$ sample $\rightarrow$ monochromator $\rightarrow$ detector

FT Raman spectrometer: source $\rightarrow$ sample $\rightarrow$ interferometer $\rightarrow$ detector

Source: Because Raman scattering is very inefficient and Stoke's shifts are less than 100 nm , a very intense line source is needed. Lasers fit the requirement perfectly. Commonly used lasers (in order of increasing wavelength) are the argon ion ($488, 514 \text{ nm}$), the krypton ion (647 nm), the He/Ne (638 nm),

the diode (700-900 nm), and the Nd/YAG (neodymium/yttrium aluminum garnet)(1030 nm). Shorter wavelengths generate Raman scattering more efficiently, but tend to photo-decompose the sample, and tend to excite fluorescence (see section XIVA, or ch. 15 of SH&N). Fluorescence is a severe interference for observing Raman spectra. Modern instruments favor the diode laser as the light source.

Samples: can be a solid, liquid or gas. Aqueous solutions are excellent matrices because water is a weak Raman scatterer (by comparison, IR spectra in water are very difficult to obtain because water is a strong IR absorber). The sample holder may have provisions for moving the sample relative to the focus of the laser beam to reduce photochemical damage. The laser beam can be focused to a small spot on a solid surface and rastered, creating a map of compounds on the surface. Fiber optics can transmit the excitation light to a remote location and collect the Raman scattered light. Scattered light is collected at an angle with respect to the incident beam to reduce the Rayleigh scattering and focused onto the entrance slit of a monochromator or collimated and directed into an interferometer.

Monochromators: usually a double or triple monochromator design because of the need for very low stray light. This is usually combined with a CCD detector for simultaneous detection of all wavelengths (multiplex advantage). We are talking big bucks here.

Interferometers: Michelson type interferometers are combined with a fast germanium photodiode, cooled to liquid nitrogen temperatures.

Review

Definitions: Stoke's and anti-Stoke's lines; Rayleigh scattering; Raman scattering.

Concepts: Explain Raman scattering in terms of an energy state diagram. Compare selection rules of Raman and IR spectroscopy. Why is water a good sample matrix for Raman spectroscopy? Sketch a complete dispersive Raman spectrometer with a double monochromator and PMT detector; show the light path and how Raman scattering is collected from the sample. Why are lasers the preferred light source? What are the advantages and disadvantages of using lasers of longer wavelength?

Calculations: Stoke's and anti-Stoke's lines in nm or cm^{-1} .

XIV. MOLECULAR LUMINESCENCE SPECTROSCOPY.

Reading: Harris, ch. 18, pp. 421-426. See also pp. 178, 433-434, 445, 450 for applications of fluorescence spectroscopy.

SH&N, ch. 15, pp. 355-371.

General points: Fluorescence (NOTE SPELLING! Flour is ground wheat!) and phosphorescence spectroscopies are among the most sensitive of instrumental methods. Detection of single molecules is possible in favorable cases. The methods also exhibit wide dynamic ranges. Relatively few molecules fluoresce or phosphoresce, so the methods are highly selective. Almost all fluorescence and phosphorescence occurs in the UV-VIS domain.

A. Fluorescence and Phosphorescence.

Definitions:

Luminescence - emission of light following excitation of the molecule by light, e^- transfer, chemical reaction, etc.

Fluorescence - luminescence without a change in spin multiplicity.

Phosphorescence - luminescence with a change in spin multiplicity.

Most commonly, fluorescence and phosphorescence follow excitation of a molecule by light. Sketches of the energy state diagram will be given during lecture. Absorption of a photon (excitation) raised the molecule from the S_0 state (ground electronic state) to an excited singlet state. In condensed phases (liquids, solids), energy is rapidly lost to the environment as heat until the molecule reaches the S_1 state (internal conversion). Some molecules undergo intersystem crossing ($S_1 \rightarrow T_1$). Photon emission is always from the ground vibrational level of the lowest excited state (S_1 or T_1). Fluorescence refers to photon emission from an excited singlet state with no spin change ($S_1 \rightarrow S_0$), phosphorescence to emission from an excited triplet state with spin change ($T_1 \rightarrow S_0$). Because some of the excitation energy is lost, fluorescence occurs at longer wavelengths (lower wavenumbers) than excitation, and phosphorescence at even longer wavelengths (sketch). The fluorescence emission spectrum ($S_1 \rightarrow S_0$) tends to be a mirror image of the lowest electronic absorption band ($S_0 \rightarrow S_1$).

The population of excited states decays exponentially after the excitation light has been turned off, hence:

$$F = F_0 e^{-t/\tau}$$

where F is the fluorescence intensity, F_0 is the fluorescence intensity at the moment the excitation light has been turned off, and τ = emission lifetime. For fluorescence, $\tau \approx 10^{-9} - 10^{-7}$ s; for phosphorescence $\tau \approx 10^{-3} - 10^{+1}$ s (because $T_1 \rightarrow S_0$ is a spin-forbidden transition, and therefore has a low probability of occurring). It is possible to distinguish fluorescence from phosphorescence by measuring the emission lifetime.

Define fluorescence quantum yield $\Phi_f = (\# \text{ photons emitted})/(\# \text{ photons absorbed})$ (the denominator is actually the # of molecules that are excited). Range: $0 \leq \Phi_f \leq 1$

We will consider now molecules dissolved in solution. Phosphorescence is rare (synthetic gem diamonds exhibit phosphorescence!), and will not be discussed further.

Factors that affect Φ_f .

9. Molecular flexibility: very flexible molecules tend to lose their excitation energy by internal conversion (loss of energy as heat, not as a photon); hence, Φ_f is essentially zero. Rigid molecules, especially aromatic systems, usually fluoresce.
10. Heavy atoms (e.g., Br, I) promote intersystem crossing ($S_1 \rightarrow T_1$), leading to a decrease in Φ_f .
11. Increasing the viscosity of the solvent, or lowering its temperature, increases Φ_f (fewer molecular collisions). Some solvents (e.g., ethanol) can be frozen with liquid nitrogen into clear glasses, in which many molecules exhibit intense fluorescence and even phosphorescence.
12. Paramagnetic molecules (e.g., O_2) promote intersystem crossing, decreasing Φ_f . Removal of dissolved O_2 (by vacuum degassing, or bubbling Ar through the solution) improves Φ_f .

B. Analytical Measurements.

Quantitative analysis:

$$F = kP_0(1 - 10^{-A})\Phi_f$$

The amount of fluorescence emitted by a solution depends on (a) the intensity of the incident light P_0 , (b) the amount of light absorbed by the solution ($1 - 10^{-A}$), where A is the absorbance of the solution, (c) the fluorescence quantum yield of the analyte Φ_f , and (d) instrumental factors k .

For optically dilute samples ($A < 0.05$), $1 - 10^{-A} \approx 2.3A$, so:

$$F = 2.3kP_0\Phi_fA = 2.3kP_0\Phi_f\epsilon bC \quad (\text{important equation!})$$

Note the following points:

1. The fluorescence intensity is proportional to concentration ($F \propto C$). Linear calibration curves extend over orders-of-magnitude in concentration. Blank signals (and hence the LOD) are largely determined by (i) fluorescence of impurities in the solution or in the cuvet walls, (ii) stray light reaching the detector, and (iii) noise of the detector.
2. The fluorescence intensity is proportional to the intensity of the excitation light ($F \propto P_0$). Brighter excitation sources (e.g., lasers) lower the LOD (subject to fluorescence of impurities).
3. The fluorescence intensity is proportional to the molar absorptivity ($F \propto \epsilon$).

Compare fluorescence spectroscopy with UV-VIS absorption spectroscopy. Absorbance is obtained from a ratio of intensities ($A = -\log(P/P_0)$). Increasing the light intensity does not affect the absorbance. As concentrations decrease, $A \rightarrow 0$, and $P \rightarrow P_0$. Precision in conc. measurement decreases rapidly below $A = 0.1$. Hence, the dynamic range for absorption spectroscopy is limited to about 2 orders-of-magnitude ($0.02 < A < 2$). Fluorescence spectroscopy can have a much larger dynamic range because the signal is a single intensity and not a ratio of intensities. LOD's for fluorescence spectroscopy are typically 1-3 orders-of-magnitude smaller than for UV-VIS absorption spectroscopy.

Ex. quinine absorption spectrum, emission spectrum and excitation spectrum.

Qualitative analysis:

See the handout (quinine spectra). A plot of F vs λ_{em} (emission wavelength) is called an emission spectrum, and is useful in identifying the analyte. A plot of F vs λ_{ex} (excitation wavelength) is called an excitation spectrum. Since the fluorescence intensity is dependent on the wavelength of the excitation light through the molar absorptivity ϵ , the excitation spectrum resembles the absorption spectrum of the analyte. However, the resemblance is distorted by the wavelength dependence of the exciting light (P_0 depends on the excitation wavelength). Having both an emission and an excitation spectrum greatly improves the chances of identifying the analyte. Also, one can choose the highest excitation λ in the excitation spectrum and the highest emission λ in the emission spectrum to do quantitative analysis with the highest possible sensitivity.

C. Fluorescence Instrumentation.

The block diagram: Source $\rightarrow \lambda_{ex}$ select $\rightarrow$ cuvet $\rightarrow \lambda_{em}$ select $\rightarrow$ em. detector

ex. detector

Fluorescence is emitted in all directions. Fluorescence is usually observed at right angles to the excitation light to minimize stray light reaching the detector. Instruments are inherently single beam in design, so a second (excitation) detector is used to directly monitor the excitation beam intensity. Signal from this

detector is divided into the signal from the emission detector to compensate for any fluctuations in excitation intensity.

Spectrofluorometer parts:

- (a) High pressure Xe arc.
- (b) Scanning excitation monochromator.
- (c) Cuvet made of silica or Pyrex (low fluorescence grade).
- (d) Scanning emission monochromator.
- (e) Detector - PMT. A Si photodiode monitors the excitation intensity.

This instrument is capable of measuring quinine (a highly fluorescent substance used as a flavoring in tonic water; often used as a fluorescence standard) concentrations down to 0.1 ppb (about 10^{-10} M).

All fluorescence measurements suffer from inner filter effects, which happen in the cuvet. Either the sample absorbs excitation light too strongly (not optically dilute) or the emission is re-absorbed before leaving the cuvet. Inner filter effects lead to negative deviations of the calibration curve at high concentrations.

Review

Definitions: luminescence, fluorescence; phosphorescence; singlet and triplet states; lifetime; internal conversion, intersystem crossing; quantum yield; excitation and emission spectra.

Concepts: Explain fluorescence and phosphorescence in terms of an energy state diagram. How do you distinguish fluorescence and phosphorescence in terms of wavelengths and lifetimes? What factors affect the fluorescence quantum yield? What types of molecules tend to exhibit fluorescence? Under what condition is fluorescence intensity linear with analyte concentration? What causes deviations in the calibration curve at high and low concentrations? Why is fluorescence so sensitive a method compared to absorption spectroscopy? Sketch a spectrofluorometer with 2 monochromator. What are inner filter effects? Why does the excitation spectrum resemble (but not match) the absorption spectrum of the molecule?

Calculations: The usual calibration curves or standard addition problems.

XV. ATOMIC ABSORPTION SPECTROSCOPY.

Reading: Harris, ch. 21, pp. 495-500, 502-505, 509-510

SH&N, ch. 8, pp. 192-202 (fundamentals of atomic spectroscopy; sample introduction methods), ch. 9, pp. 206-218, 220-224 (flame and electrothermal atomization, hollow cathode lamp, atomic absorption spectrometers, interferences in AAS, calibration curves and standard addition).

Atomic spectroscopy methods are based on light absorption and emission (via electronic transitions, all in the UV-VIS domain) of atoms in the gas phase. The goal is elemental analysis - identity and concentration of a specific element in the sample; chemical and structural information are lost. The sample is destroyed.

A. Atomic Spectra.

Atoms in the gas phase have very well defined electronic energy levels for the electrons. Consequently light is absorbed by a ground state atom or emitted by an excited atom at very precise wavelengths,

resulting in line spectra. In the UV-VIS domain, each element has hundreds to thousands of absorption/emission lines. The bandwidth (FWHM) of each line is extremely narrow (ca. 0.002 to 0.005 nm; bandwidth increases with temperature and pressure). Consequently, it is possible to selectively detect and quantify one specific element by carefully setting the observation wavelength and keeping the observation bandpass very small. Certain electronic transitions have the highest probability; the corresponding absorption/emission lines are called resonance lines, and have the best wavelengths for analysis of that element (Ex. 589 nm for Na, 248.3 nm for Fe). The absorption/emission spectrum of an atom changes dramatically if it is ionized. The absorption spectrum also changes if the atom is in an excited state.

The goal in atomic absorption spectroscopy: From a sample in solution (used because solutions help to reduce matrix effects), generate the elemental gaseous atoms in their ground state at a concentration proportional to the concentration of the element in the sample. The concentration of the gaseous atoms is measured by absorption spectroscopy using Beer's law. There are two commonly used methods for achieving this goal: flame AAS and the electrothermal method.

B. Flame Atomic Absorption Spectroscopy (Flame AAS).

The most common flame method is based on a nebulizer and a slot burner. Oxidant gas causes the nebulizer to draw up the liquid sample and produce a spray of tiny droplets. The larger droplets strike the walls and baffles and go down the drain; most of the sample is lost! The oxidant and fuel are pre-mixed before entering the slot burner. The slot burner produces a linear flame 5-10 cm long. Desirable attributes of the nebulizer and burner include:

- (a) Production of small & reproducible droplets.
- (b) No memory effects (good drainage).
- (c) Production of a quiet and stable flame.
- (d) Prevention of flashback.

Gas pressures and flow rates must be carefully controlled. The choice of fuel and oxidant is limited. The most common combinations are: acetylene - air ($T_{\max} = 2400$ K) and acetylene - nitrous oxide ($T_{\max} = 2800$ K). Fuel/oxidant ratios can be adjusted to be stoichiometric (hottest flame), reducing or rich (excess fuel helps to reduce metal oxides), or oxidizing or lean (excess oxidant helps to break down organics).

The flame must (a) evaporate the droplets, (b) decompose the organics and salts to atoms and (c) reduce ions to atoms. The atom concentration varies with height above the burner because of the complex flame chemistry; different heights are appropriate for different elements.

C. Electrothermal Atomization (Graphite Furnace Atomic Absorption).

Use a graphite furnace - a graphite tube heated by electrical current.

Operation (usually done via computer control).

1. Inject the sample (5-50 μ L) into the sample cup.
2. Dry the sample (100°C).
3. Ash the sample (500-1000°C) to break down the organics.
4. Atomize the sample by ramping the temperature rapidly to 2000-2500°C. For a fraction of a second, a plume of atoms fills the tube; the absorbance measurement must be made during this time.

- Clean the sample cup by heating to 3000°C and then cool to room temperature.

The total time per sample is typically about 3 minutes.

Comparison of the flame and graphite furnace atomization methods:

Flame: better precision, better accuracy, more rapid analyses of samples, less expensive equipment.

Furnace: greater sensitivity, much lower LOD, uses much less sample.

D. Atomic Absorption Spectrometers.

Assumptions: (i) Beer's law holds for the atoms in the flame or graphite furnace, and (ii) the concentration of atoms in the flame or furnace is proportional to the concentration of analyte in the sample.

The block diagram:

Line source → chopper → flame/furnace → monochromator → detector

- Line Source: a hollow cathode lamp (HCL). The HCL consists of a hollow cup cathode lined with the desired element and an anode in a low pressure argon atmosphere. Application of several hundred volts causes electrons to pass through the argon, forming argon cations (Ar^+). The argon ions blast atoms of the element into the gas phase and excite them by collisions. Consequently, the HCL emits lines of the element coating the cathode. To change the element analyzed, one must change the HCL (although some HCL's are coated with several elements). HCL's are often mounted on a turret so lamps can be switched easily. The bandwidth of one emitted line is roughly 3 times smaller than the bandwidth of the absorption in the flame or furnace because the HCL operates at near room temperature and low pressure. Beer's law is generally valid for Abs. up to 0.8 (the upper limit of linearity in calibration curves). Question: why not use a continuum source and a good monochromator rather than a HCL?
- Chopper: Light emitted by the flame or graphite furnace (including emission by the analyte atoms) is stray light. So the HCL light is modulated by the chopper and the Amp/Processor synchronously detects the signal due to the HCL light. Light emitted by the flame/furnace is ignored. In a quasi-double beam design, the chopper sends the light in a path around the flame/furnace to provide P_0 .
- Monochromator: (a) isolates one spectral line from the HCL lines (a bandpass of 0.2 nm is usually sufficient to isolate 1 line); (b) prevents most of the flame or graphite furnace emission from reaching the detector. Because the dynamic range of calibration curves is so limited (ca. 1 order-of-magnitude), it is convenient to switch to another line of the element to change the sensitivity of the method.
- Detector: a PMT.

To obtain an absorbance measurement:

- Run a blank (e.g. pure water). The instrument records P_0 or adjusts PMT sensitivity to get a preset P_0 .
- run a sample. The instrument calculates and displays A .

Most modern instruments are automated with computer control and data acquisition. Both calibration curves (Beer's law plots) and standard addition methods are used.

Review

Definitions: resonance line

Concepts: What are the two goals in atomic absorption spectroscopy? Sketch a nebulizer/slot burner; describe how it works. Sketch a hollow cathode lamp and discuss how it works. Why is a line source needed for AAS? Sketch a graphite furnace and describe the temperature program of a graphite furnace and what happens at each temperature. Sketch a block diagram of an atomic absorption spectrometer. What is the role of the chopper and the monochromator? Compare flame and graphite furnace AAS.

Calculations: The usual calibration curves or standard addition problems.

Note: Sections E (Chemical interferences) and F (Spectroscopic interferences) will not be covered in lecture. You are not responsible for knowing this material; this is for your information.

- E. Chemical interferences - affect the concentration of ground state atoms in the flame/furnace.
1. Formation of stable compounds i.e. refractory oxides (WO , CaAlO_2) or refractory salts (CaPO_4). Possible cures: use hotter temperatures to break apart the compounds; use a rich flame to reduce metal oxides; avoid certain anions with certain analyte cations; chelate the metal analyte.
 2. Ionization ($\text{M} \rightarrow \text{M}^+ + \text{e}^-$). Especially a problem for Group I metals which have low ionization energies. Cure: raise the free electron concentration in the gas phase by adding a large excess of Cs salt (ionization suppressant) to the sample; the excess e^- from the Cs atoms suppress ionization of other metal atoms.
 3. Excitation ($\text{M} \rightarrow \text{M}^*$). M^* does not absorb light at the same wavelengths as M. Cure: lower the temperature to decrease the fraction of atoms that are excited.
- F. Spectroscopic Interferences - prevents accurate absorbance measurements.
1. Molecular absorption - molecules (e.g. OH) in the flame or furnace have broad absorption bands which often overlap with the desired atomic line.
 2. Light scattering by particles (smoke!). Especially a problem for samples with high salt content, as the salt tends to not atomize completely. The effect is the same as for molecular absorption; the desired analyte absorbance is on top of a broad apparent absorption. Graphite furnace measurements can be improved by carefully ashing the sample before atomization.

XVI. ATOMIC EMISSION SPECTROSCOPY

Reading: Harris, ch. 21, pp. 501-502, 505-507
SH&N, ch. 10, pp. 230-244.

The sample is converted into excited gaseous atoms and ions which emit light at characteristic wavelengths (wavenumbers). The analyte is identified by emission at a known wavelength (qual. analysis) and its concentration is determined from the intensity of the emission at that wavelength (quant. analysis). Historically the oldest type of analytical spectroscopy. Used to analyze composition of stars.

- A. Atomic Spectra Revisited.

Atoms or ions in the gas phase can be excited by high temperatures or by light absorption. A fraction of the atoms or ions lose their excitation energy by giving off a photon. The most intense emission line for a neutral atom is the resonance line (see XV.A). Self-absorption reduces the emission signal and leads to non-linearity of calibration curves. It is caused by the light emitted from excited atoms passing through ground-state atoms.

B. Atomic Emission Spectrometers.

hot plasma source → monochromator → detector

Emission sources: The source must vaporize the sample, break down all compounds to atoms and ions, and excite the atoms and ions. Very high temperatures are required, much higher than in atomic absorption. Historically, electrical arcs (low voltage, high current), electrical sparks (high voltage, low current), and flames have been used to atomize and excite the analytes. However, these have largely been replaced by 2 plasma sources, the ICP torch and the DC plasma jet. Of these two, the ICP torch is the most important. Like the flame AA experiment, the sample is introduced into the instrument as a solution fed into a nebulizer, creating a fine spray of droplets in a flowing gas stream.

Inductively coupled plasma (ICP): A plasma (partially ionized gas) of argon is formed and sustained by intense electric and magnetic fields inside a coiled radio antenna (27 MHz, 1-2 kW power). A triple-tube quartz torch is used to (a) generate an annular plasma, (2) cool the outer edges of the plasma (thermal pinch) and (3) introduce the sample as droplets in an Ar gas stream. Temperatures are about 10,000 K at hottest part of plasma. Observation is made 1-3 cm above hottest part; local temperatures are still high (ca. 6000 K). Most analytes (usually metals) exist as ions at this temperature.

Advantages: (a) a very wide dynamic range (3 to 6 orders-of-magnitude) on calibration curves; (b) stable operation over long periods of time, (c) almost no chemical interferences due to the very high temperatures.

Disadvantages: (a) expensive to buy and operate; (b) rapid consumption of high purity Ar gas (several L/min). All plasma sources have one basic disadvantage - the emission intensity is quite sensitive to fluctuations in the plasma temperature.

C. Monochromators/ Polychromators.

A polychromator has multiple exit slits with multiple PMT detectors or a CTD - see color plate 22 in Harris. Because there are many emission lines from the plasma, often separated by only a few Angstroms, high resolution is required in monochromators and polychromators. Designs are based on an echelle grating operating at high orders combined with an order-sorting prism (diagram, see color plate 23 in Harris)), or on concave gratings and the Rowland circle (a circle whose diameter equals the radius of curvature of the grating) (diagram). The entrance and exit slits and the grating are all on the Rowland circle. For high R, the monochromator must be large; f.l.'s of 0.5 meters and up are common.

D. Detectors.

Usually a PMT (multiple PMT's in a polychromator) or CTD is used. The CTD is combined with an echelle grating monochromator. Regions on the CTD can be assigned to particular wavelengths associated with particular elements. Both the polychromator + multiple PMTs and the echelle with CTD can monitor concentrations of 10 or more elements from one sample within a few minutes. These devices are used heavily by the metallurgy industry to monitor composition of alloys.

Because AES can easily perform multi-element analysis, an internal standard (an element not naturally found in the samples) is often incorporated at a constant concentration into each sample. To compensate for fluctuation of plasma emission intensity (caused by fluctuations in plasma temperature), the emission intensity of each element line is divided by the emission intensity of the internal standard.

Review

Definitions: self-absorption; internal standard.

Concepts: Sketch a block diagram of an atomic emission spectrometer. Sketch an ICP torch, discuss how it works, and list its advantages/disadvantages. Sketch a AES instrument based on either a Rowland circle monochromator with multiple PMTs or an echelle monochromator with an order-sorting prism and a CTD. Why is an internal standard used in AES?

Calculations: The usual calibration curves or standard addition problems.

XVII. FUNDAMENTALS OF ELECTROCHEMISTRY.

Reading: Harris, ch. 14, pp. 283-305, ch. 15, pp. 314-317

SH&N, ch. 22, pp. 564-582, 587-588; ch. 23, pp. 591-594 (reference electrodes)

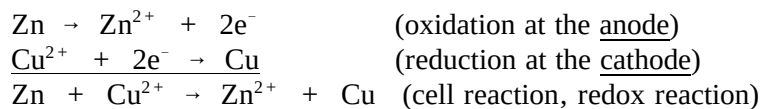
The electrochemical instrumental methods we will cover include potentiometry, voltammetry and coulometric methods. To better understand these methods, it is useful to review basic concepts in electrochemistry.

A. The Electrochemical Cell.

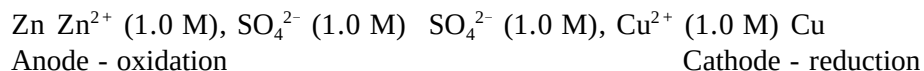
Consider the Daniel's cell: Zn electrode in ZnSO₄ solution, Cu electrode in CuSO₄ solution, plus a salt bridge. The essential components of a cell are:

- Two half-cells, each containing an electrode and electrolyte of a half-reaction.
- A salt bridge, a path for ion flow between the 2 half-cells that inhibits bulk mixing.

A voltmeter connected to the 2 electrodes will display 1.1 V, with the Cu electrode positive. If the electrodes are connected, then current will flow. In the external circuit, electrons move from the negative Zn electrode to the positive Cu electrode. In the electrolyte and salt bridge, anions will flow from Cu to Zn and cations in the opposite direction. At the electrode/electrolyte interfaces, 2 half-reactions will occur:



Cell notation: electrode electrolyte | electrolyte electrode, where | = phase boundary, || = salt bridge, and by convention the left electrode is the anode ("-" for galvanic cell, "+" for electrolysis cell). For the Daniel's cell we write:



By convention, the observed cell potential ΔE is divided into 2 half-cell potentials, each assigned to one half rxn:

$$\Delta E = E_{\text{right}} - E_{\text{left}} = E_{\text{cat}} - E_{\text{an}} \quad (= E(\text{Cu}^{2+}/0) - E(\text{Zn}^{2+}/0) \text{ for the Daniel's Cell})$$

B. Cell Potentials and the Nernst Equation.

Define standard state as the condition of unit activity of a substance (pure solid, pure liquid, gas at 1 atm, solution of 1 M activity). A superscript "0" signifies standard state. The activity of substance X = $A_x = \gamma_x[X]$, where γ_x is the activity coefficient and [X] is the molar concentration of X.

Each half-cell potential can be calculated using the Nernst equation for half-rxns:

$$E = E^0 - (2.3RT/nF)\log(Q) = E^0 - (0.05916 \text{ V}/n)\log(Q)$$

where Q = reaction quotient when the half-rxn is written as a reduction; n = # of e^- transferred in the half-rxn; and E^0 = standard potential of the half-rxn with respect to the SHE (the Standard Hydrogen Electrode). A Table of Standard Potentials shows the half-rxn listed as a reduction.

Ex. Write the Nernst equ. for the following half-reactions:

$$\begin{array}{ll} \text{(a) } \text{Pb}^{2+} + 2e^- \rightleftharpoons \text{Pb(s)} & E = E^0 - (0.05916/2)\log(1/[\text{Pb}^{2+}]) \\ \text{(b) } \text{Fe}^{3+} + e^- \rightleftharpoons \text{Fe}^{2+} & E = E^0 - (0.05916/1)\log([\text{Fe}^{2+}]/[\text{Fe}^{3+}]) \\ \text{(c) } \text{AgCl(s)} + e^- \rightleftharpoons \text{Ag} + \text{Cl}^- & E = E^0 - (0.05916/1)\log([\text{Cl}^-]) \end{array}$$

If activity coefficients are factored out of the reaction quotient in the Nernst equation, then the standard potential is replaced with the formal potential ($E^{0'}$), for which solution concentrations must be specified. Molar concentrations are used in the reaction quotient.

Question: What is ΔE^0 for the Daniel's Cell given the standard potentials $E^0(\text{Cu}^{2+}/0) = +0.337 \text{ V}$ and $E^0(\text{Zn}^{2+}/0) = -0.763 \text{ V}$?

C. Reference Electrodes.

Definition: a half-cell which has a constant potential when used in an electrochemical cell. It is used as a reference point on the potential scale for all electrochemical measurements. In most cases, the reference electrode is the anode (REF = analyte half-cell). There are 3 important ref. electrodes:

1. Standard Hydrogen Electrode (SHE)(sketch).

Half-rxn: $2\text{H}^+ + 2e^- \rightleftharpoons \text{H}_2(\text{g})$ Cell notation (anode): $\text{Pt} * \text{H}^+ (1 \text{ M}), \text{H}_2 (1 \text{ atm}) **$
or, for short, SHE

With all components in their standard state ($\text{pH} = 0$ ($A_{\text{H}^+} = 1 \text{ M}$), $P_{\text{H}_2} = 1 \text{ atm}$), $E^0 = 0 \text{ V}$ at all temperatures by definition. This type of reference electrode is rarely used in practice.

2. Saturated Calomel Electrode (SCE)(sketch).

Half-rxn: $\text{Hg}_2\text{Cl}_2(\text{s}) + 2e^- \rightleftharpoons 2\text{Hg(l)} + 2\text{Cl}^-$ Cell notation (anode): $\text{Hg(l)} * \text{Hg}_2\text{Cl}_2(\text{s}), \text{Cl}^- (3.5 \text{ M}) **$
calomel or, for short, SCE

With saturated KCl (3.5 M) as the electrolyte, $E_{\text{SCE}} = +0.241 \text{ V}$ vs SHE at 25°C.

3. Silver/Silver Chloride (Ag/AgCl)(sketch).

Half-rxn: $\text{AgCl(s)} + \text{e}^- \rightarrow \text{Ag} + \text{Cl}^-$ Cell notation (anode): $\text{Ag} \mid \text{AgCl(s)}, \text{Cl}^- (3.5 \text{ M})$ **
or, for short, Ag/AgCl

The chloride activity is fixed by using saturated KCl or a lower concentration of KCl. If saturated KCl is used, then $E_{\text{Ag/AgCl}} = +0.197 \text{ V}$ at 25°C .

Problem: If $E^\circ(\text{Pb/Pb}^{2+}) = -0.126 \text{ V}$ vs SHE, what is its E° vs SCE? What is the cell notation?

$$-0.126 \text{ V} = E^\circ - E_{\text{SHE}} \quad (\text{combine the 2 equations to cancel } E_{\text{SHE}} \text{ and find } E^\circ - E_{\text{SCE}})$$

$$\underline{-(+0.241 \text{ V} = E_{\text{SCE}} - E_{\text{SHE}})}$$

$$(-0.126 \text{ V}) - (+0.241 \text{ V}) = -0.367 \text{ V} = E^\circ - E_{\text{SCE}}$$

Cell notation: SCE $\mid \text{Pb}^{2+} (1 \text{ M}) \mid \text{Pb}$

D. Liquid Junction Potentials.

A liquid junction potential (E_{lj}) develops at the contact point of two dissimilar electrolytes e.g. at salt bridges. The E_{lj} is part of the total cell voltage, but its sign and magnitude are generally not known. In the worst cases (e.g. very different pH's), E_{lj} can reach ca. $\pm 30 \text{ mV}$. It can be kept to a minimum (about $\pm 1 \text{ mV}$) by using a very high concentration of an equitransferent (equal current carried by the cations and the anions) salt (e.g. KCl) on one side of the junction; hence salt bridges and reference electrodes often use saturated KCl electrolytes.

Major consequence: In nearly every cell voltage, there is an uncertainty of at least $\pm 1 \text{ mV}$ due to the unknown liquid junction potential.

$$\Delta E = E_{\text{cat}} - E_{\text{REF}} \pm E_{\text{lj}}$$

This affects the precision of potentiometry considerably.

Review

Definitions: Oxidation & reduction; anode & cathode; half-cells, half-reactions and cell (redox) reactions; salt bridges; standard & formal potentials; reference electrode; liquid junction potential.

Concepts: Convert cell notation into 2 half-reactions and the cell reaction. Write the Nernst equation for a half-reaction. What are the 3 major types of reference electrodes, what is their construction, and what are their half-reactions? What limitation is imposed by the liquid junction potential?

Calculations: Calculating half-cell and cell potentials using the Nernst equation. Conversion of standard or formal potentials to different reference electrode scales.

XVIII. POTENTIOMETRY.

Reading: Harris, ch. 15, pp. 317-340

SH&N, ch. 23, pp. 594-618 (omit “electrodes of the third kind”, and “ISFETs”).

The most widely used electrochemical method.

Advantages: fast, selective, cheap, non-destructive, noncontaminating, capable of measuring concentrations of analytes over many orders-of-magnitude, unaffected by color or turbidity. Can measure concentrations in very small volumes. Can be used in-situ in living systems.

Disadvantages: useful for quantitative analysis only, accuracy and precision are not as good as most spectroscopic measurements.

E. General Principles.

Block diagram: ref. electrode sample indicator electrode pH meter

The sample is incorporated into an electrochemical cell containing a reference electrode and an indicator electrode. The indicator (IND) electrode responds to the analyte; the reference (REF) electrode provides a constant reference potential. The pH meter (a specialized voltmeter) measures the cell potential ΔE between 2 electrodes at zero current.

$$\Delta E = E_{\text{ind}} - E_{\text{ref}} + E_{\text{lj}} \quad (1)$$

E_{lj} is the liquid junction potential (see XVII.D) which is present in virtually every cell. The 2 basic indicator electrodes are (1) metallic electrodes and (2) ion-selective electrodes (ISE's). Metallic electrodes will be covered briefly; the main focus will be on ISE's. Equ. 1 is transformed into the following POTENTIOMETRY EQUATION:

$$\Delta E = K + S \cdot \log(a_A) \quad (2)$$

where K is the calibration constant, S is the slope, and a_A is the activity of the analyte A . The units of ΔE , K , and S are volts (V), although millivolts (mV) are frequently used. Warning: make sure that these 3 variables have the same units in your calculations! K is determined by measuring one or more standards. S can be calculated theoretically (see below), or measured experimentally using 2 or more standards.

Once K and S are known, then equ. 2 can be inverted to convert a sample ΔE to calculate activity (or conc.):

$$\log(a_A) = (\Delta E - K)/S \quad (3)$$

Often, $\log(a_A)$ is replaced with pA ($= -\log(a_A)$). Then equ. 2 becomes $\Delta E = K - S \cdot pA$, and equ. 3 becomes $pA = -(\Delta E - K)/S$.

B. Calibration Methods.

First, note that this is the only instrumental method discussed in which the signal is NOT linear with respect to analyte concentration. The signal is linear with the log of the activity.

If calibrations are done under conditions that hold the activity coefficients constant, the activity of A (a_A) can be replaced by the molar conc. of A ($[A]$). To achieve this goal, both samples and standards are mixed with an total ionic strength adjusting buffer (TISAB) (a concentrated salt solution) to reach a high and constant ionic strength. Note: in most calculations, we will ignore activity effects and use molar concentrations. Calibration methods are required for two reasons:

- (a) The uncertainty in K (at least ± 1 mV) introduced by the liquid-junction potential E_{lj} .
- (b) Non-ideal S values (often smaller in magnitude than the theoretical values).

1. Single point calibration: measure 1 standard and 1 sample. The slope S is assumed to be ideal (i.e., have its theoretical value). Set up two equations, one for the standard and one for the sample. Solve for K using the standard. Insert the K value into the sample equation and solve for the unknown analyte concentration.

Ex. A chloride ISE and SCE immersed in a 0.010 M chloride solution yielded a cell potential of -0.159 V. In a sample, the cell potential was -0.201 V. A chloride ISE has a theoretical slope of -0.05916 V. Calculate pCl and $[Cl^-]$.

$$-0.159 = K - 0.05916 \cdot \log(0.010) = K - 0.05916(-2.00) = K + 0.11832; K = -0.159 - 0.118 = -0.277 \text{ V}$$

$$-0.201 = -0.277 - 0.05916 \cdot \log([Cl^-]); \log([Cl^-]) = ((-0.201) - (-0.277))/(-0.05916) = -1.28$$

$$pCl = -\log([Cl^-]) = +1.28; [Cl^-] = 10^{-1.28} = 0.052 \text{ M}$$

2. Calibration curve: plot ΔE vs $\log([A])$ for a series of standards. Fit the data to a linear regression line of slope S and intercept K. If only 2 standards are given, solve for S and K using 2 equations. Use S and K to calculate concentrations of any samples. Non-linear parts appear at high conc. (activity effects) and at low conc. (the indicator electrode responds to other ions in solution).

Ex. A Ca ISE + ref. electrode were standardized in 2 solutions, and then immersed in a blood serum sample to determine Ca^{2+} . From the following data, calculate K, S, and $[Ca^{2+}]$ for the blood serum sample.

$[Ca^{2+}]$	ΔE
3.0 mM	-18 mV
30. mM	$+9$ mV
$[Ca?]$	-4 mV

Two equations: $-18 = K + S \cdot \log(0.0030)$ and $+9 = K + S \cdot \log(0.030)$

Subtract equations: $-27 = (-2.52)S - (-1.52)S = (-1.00)S$; $S = +27$ mV (ideal S is $+29.6$ mV)

Substitute: $-18 = K + 27(-2.52) = K - 68$; $K = +50$ mV

Sample: $-4 = +50 + 27 \cdot \log([Ca?]); \log([Ca?]) = (-4 - 50)/(+27) = -2.0$;

$[Ca?] = 10^{-2.0} = 0.010 \text{ M or } 10. \text{ mM}$

3. Standard addition: measure sample, add an aliquot of a standard solution to the cell without moving the electrodes, and remeasure sample. The slope S is assumed to be ideal. Set up two equations and solve for the analyte concentration; do NOT neglect dilution!. Eliminate K by subtraction and solve for the sample conc. The advantage of this method is that the 2 electrodes can remain in the sample, ensuring that E_{lj} is as constant as possible (minimum uncertainty in K).

Ex. A 25.0 mL volume of fluoridated tapwater was analyzed using a fluoride ISE (ideal $S = -0.05916$ V). The measured potential was $+0.4524$ V. 10.0 mL of $5.00 \times 10^{-4} \text{ M } F^-$ was added to the sample and mixed. The new potential was $+0.4336$ V. Calculate the molar fluoride concentration and convert it to ppm fluoride.

$$+0.4524 = K - 0.05916 \cdot \log\{[F^-]\}$$

$$+0.4336 = K - 0.05916 \cdot \log\{[F^-](25.0/35.0) + (5.00 \times 10^{-4})(10.0/35.0)\}$$

Subtract, rearrange, and solve:

$$(+0.4524) - (+0.4336) = +0.0188 = +0.05916 \cdot \log\{[F^-](25.0/35.0) + (5.00 \times 10^{-4})(10.0/35.0)\} - 0.05916 \cdot \log\{[F^-]\}$$

Recall that $\log(a) - \log(b) = \log(a/b)$.

$$+0.0188 = +0.05916 \cdot \log\{([F^-](25.0/35.0) + (5.00 \times 10^{-4})(10.0/35.0))/[F^-]\}$$

$$+0.0188/(+0.05916) = +0.318 = \log\{([F^-](25.0/35.0) + (5.00 \times 10^{-4})(10.0/35.0))/[F^-]\}$$

$$10^{+0.318} = 2.08 = ([F^-](25.0/35.0) + (5.00 \times 10^{-4})(10.0/35.0))/[F^-]$$

$$2.08[F^-] = [F^-](0.714) + 1.43 \times 10^{-4}; [F^-](2.08 - 0.714) = 1.43 \times 10^{-4}; [F^-] = (1.43 \times 10^{-4})/1.37 = 1.04 \times 10^{-4} \text{ M}$$

$$(1.04 \times 10^{-4} \text{ mol F}^-/\text{L})(19.0 \text{ g/mol})(1000 \text{ mg/g}) = 1.98 \text{ mg/L} = 1.98 \text{ ppm fluoride}$$

Given an uncertainty in cell potential (ΔE_{err}), the % relative error in [A] is:

$$\% \text{ rel. error} = \pm 100 \cdot (10^X - 1) \quad (4)$$

where $X = \Delta E_{\text{err}}/S$. For an error of $\pm 1 \text{ mV}$ (the minimum uncertainty due to the liquid junction potential) and a S of 59 mV , the % rel. error in [A] is $\pm 4.0\%$.

C. Metallic Electrodes (this section will not be covered in lecture. It will be covered in the homework, and you will be responsible for test questions from this section)

The indicator electrode is a metal electrode that responds to a half-reaction. The half-reaction includes the analyte in the sample. The cell potential is controlled by the Nernst equation.

$$\Delta E = E_{\text{ind}} - E_{\text{ref}} \quad E_{\text{ind}} = E^0 - (2.3RT/nF)\log(Q)$$

$$\Delta E = (E^0 - E_{\text{ref}}) - (2.3RT/nF)\log(Q) = K + S \cdot \log(a_A)$$

Hence, K and S are derived from the Nernst equation. In principle, potentiometric measurements using metallic electrodes do not need calibration. We will consider only two cases.

4. Electrodes of the 1st kind - a metal electrode in contact with its cation.

Ex. A copper electrode in contact with Cu^{2+} (the analyte).

$$\text{Cu}^{2+} + 2e^- \rightleftharpoons \text{Cu} \quad E^0 = +0.337 \text{ V}$$

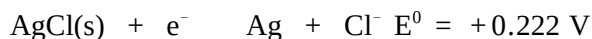
$$\Delta E = (E^0 - E_{\text{ref}}) - (2.3RT/nF)\log(Q) = (+0.337 \text{ V} - E_{\text{ref}}) - ((0.05916 \text{ V})/2) \cdot \log(1/[\text{Cu}^{2+}])$$

Since $\log(1/x) = -\log(x)$, then $-((0.05916 \text{ V})/2) \cdot \log(1/[\text{Cu}^{2+}]) = +((0.05916 \text{ V})/2) \cdot \log([\text{Cu}^{2+}])$

$$\Delta E = K + S \cdot \log([\text{Cu}^{2+}]) \text{ where } K = (+0.337 \text{ V} - E_{\text{ref}}) \text{ and } S = +((0.05916 \text{ V})/2) = +0.02958 \text{ V}$$

5. Electrodes of the 2nd kind - a metal electrode in contact with an insoluble salt and its anion.

Ex. A silver electrode in contact with AgCl(s) and Cl⁻ (the analyte)



$$\Delta E = (E^0 - E_{\text{ref}}) - (2.3RT/nF)\log(Q) = (+0.222 \text{ V} - E_{\text{ref}}) - ((0.05916 \text{ V})/1)\cdot\log([\text{Cl}^-])$$

Hence, $K = (+0.222 \text{ V} - E_{\text{ref}})$ and $S = -0.05916 \text{ V}$

Be able to derive expressions for K and S given the electrode type. Be able to calculate concentrations of analytes given the standard potential, the reference electrode potential, and the cell potential. See the homework for examples.

D. Ion Selective Electrodes (ISE).

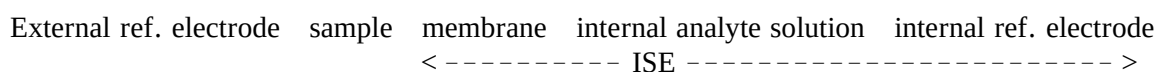
The fundamental mechanism for developing a potential related to the analyte activity is different from that of the metallic electrodes. An ISE contains a membrane which has two important properties. The membrane conducts ions, and it selectively adsorbs one ion from a contacting solution. A potential develops between the surface of the membrane and the solution on each side. The total potential across the membrane and the contacting solution is given by:

$$E_b = (2.3RT/zF)\cdot\log(a_1/a_2)$$

where E_b is the membrane potential, z is the charge of the adsorbing ion, and a_1 and a_2 are the activities of the ions in the contacting solutions. If a_2 is held constant, then the membrane potential responds to the analyte activity a_1 in a sample.

Reminder: for metal ISE's, the ideal Nernst slope ($2.3RT/nF$) contains "n", the number of electrons in the half-rxn. For ISE's, the Nernst slope ($2.3RT/zF$) contains "z", the charge of the ion being detected.

To measure the membrane potential, it is necessary to create an electrochemical cell with two reference electrodes, one on each side of the membrane. The electrochemical cell consists of a membrane, internal and external reference electrodes, an internal solution and a sample solution, both containing the analyte.



The membrane, internal analyte solution and internal reference electrode are packaged as one unit called the ion selective electrode. Only the membrane is exposed to the sample. Since the two ref. electrodes have a constant potential, the cell voltage is controlled by E_b :

$$\Delta E = K + S\cdot\log(a_A) \tag{5}$$

$$K = E_{\text{ref}}(\text{int}) - E_{\text{ref}}(\text{ext}) + E_{ij} - (2.3RT/zF)\cdot\log(a_A(\text{int})) \text{ and } S = +(2.3RT/zF) = +0.05916 \text{ V}/z \text{ (ideal value)}$$

Ex. What is the ideal slope for a Cl⁻ ISE? a Ca²⁺ ISE?

For Cl⁻, ideal $S = +0.05916 \text{ V}/(-1) = -0.05916 \text{ V}$; for Ca²⁺, ideal $S = +0.05916 \text{ V}/(+2) = +0.02958 \text{ V}$

As noted in earlier discussion, K is usually determined by a calibration. In most real ISE's, the slope S is quite often smaller in magnitude than the ideal S value listed above, and consequently it should be determined by a calibration curve.

There are 3 basic types of membranes: glass, solid-state, and liquid/polymer. All have high internal resistances, especially the glass membranes. In this course, we will only discuss the glass membrane ISEs.

Glass membranes - a thin (50 μm) glass membrane that conducts Na^+ or Li^+ ; the membrane resistance is ca. $10^8 \Omega$. The surface of the glass becomes a hydrated gel 10 nm thick after soaking in water. The gel contains $-\text{Si}-\text{O}^-$ sites that selectively adsorb monocations (H^+ , Na^+ , K^+ , Ag^+ , NH_4^+). The selectivity is controlled by the glass composition.

The most important glass membrane ISE is the pH electrode. The equation relating cell potential to pH is:

$$\Delta E = K + (2.3RT/F) \cdot \log(a_{\text{H}^+}) = K - (0.05916 \text{ V})\text{pH} \text{ (ideal } S = -0.05916 \text{ V)} \quad (6)$$

The pH electrode contains an internal solution of 0.1 M HCl and an Ag/AgCl REF electrode. In the absence of interfering ions (Na^+ , K^+ , Ag^+ , NH_4^+), its response to H^+ is Nernstian (i.e. linear calibration curve) from pH 0 to pH 14!. Calibration of pH electrodes is done by standard buffers. A combination pH electrode has the external reference electrode (Ag/AgCl) built on the outside of the pH electrode, so only one electrode assembly is immersed in the sample.

Ex. A combination pH electrode is immersed in 0.050 m KHP buffer ($\text{pH} = 4.01$ at 25°C). The cell potential was +176 mV. The electrode was transferred to a sample, where a cell potential of +100 mV was obtained. What is the pH of the sample? (This is a single point calibration; you must assume that S is ideal)

$$\Delta E = K - (59.16 \text{ mV})\text{pH}; \quad +176 \text{ mV} = K - (59.16 \text{ mV})(4.01); \quad K = 176 + (+237) = +413 \text{ mV}$$

$$+100 \text{ mV} = +413 \text{ mV} - (59.16 \text{ mV})\text{pH}; \quad \text{pH} = (100 - 413)/(-59.16) = 5.29$$

The interfering ions, especially Na^+ , produce alkaline error above pH 10. Alkaline error leads to pH readings that are lower than the true pH.

E. pH Meters and pH Measurement.

Because ISE's and especially pH electrodes have very high internal resistances, it is necessary that the voltmeter have a very high input resistance. A pH meter is first a voltmeter with an input resistance greater than $10^8 \Omega$; $10^{12} \Omega$ or higher input resistances are typical. Second, a pH meter can convert cell voltage into a display of pH. The electronic conversion is given by the equation:

$$\text{pH} = (K - \Delta E)/S \quad (7)$$

Because of variations of E_{ij} and non-ideal behavior of the glass membrane (drift in E_b , non-ideal S), it is necessary to calibrate pH electrode cells using standard buffers.

There are 3 calibration settings on a pH meter, a calibration constant (K), a slope constant (S), and the temperature (which affects S ; recall that ideal $S = +2.3RT/zF$). Modern pH meters often include a temperature probe which measures the sample temperature and corrects S for the temperature.

Recommended calibration procedure for pH measurement using 2 buffers which bracket (if possible) the sample pH. Both K and S are set by this procedure.

- (a) Immerse the pH and ext. ref. electrodes in the standard buffer closest to pH 7. Adjust the CALIBRATE control until the pH reading is at the standard buffer pH (which sets K). Some pH meters will recognize a standard buffer and automatically lock onto the correct pH.
- (b) Rinse the electrodes and immerse them in the 2nd buffer. Adjust the SLOPE control to set the readout to the known pH. Again, some pH meters will recognize the buffer, automatically lock onto the correct pH, and set the slope.
- (c) Rinse the electrodes and immerse them in the sample; read the pH.

Given an unavoidable uncertainty in the cell voltage (E_{lj}) due to liquid-junction potential:

$$\text{pH error} = E_{lj}/S = E_{lj}/(0.05916 \text{ V}) \quad (8)$$

A minimum error of ± 1 mV yields an error of ± 0.017 pH units. More precise pH measurements are very difficult to obtain. However, changes in pH in an assembled cell can be measured to a precision of ± 0.001 pH because E_{lj} is nearly constant.

Review

Definitions: Potentiometry, indicator electrodes; redox & ion-selective electrodes; total ionic strength buffer; membrane potential; interfering ions; alkaline error.

Concepts: What are the advantages and disadvantages of potentiometry? Given the potentiometric electrode, what is the theoretical slope of the calibration curve (sign and magnitude)? Briefly describe each of three methods of potentiometric analysis: single point calibration, calibration curve, standard addition; indicate when you must assume that S has the ideal value. What is the role of the membrane in an ISE? Name and briefly describe the membrane of a pH electrode. What are the two characteristics of a pH meter? Describe a calibration procedure for a pH electrode and indicate how K and S are set. What causes alkaline error? What is the effect of alkaline error on a pH reading?

Calculations: Given the ISE or indicator electrode half-reaction, calculate the concentration [X], pX or pH given data for a single point calibration, a calibration curve, or standard addition; this may include determining the value of the ideal slope S. Calculate the error in concentration or pH given the magnitude of the error in the cell voltage.

XIX. INTRODUCTION TO VOLTAMMETRIC METHODS

Reading: Harris, ch. 17, pp. 377- 378, 386-388
SH&N, ch. 22, pp. 586-588; ch. 25, pp. 639-650, 654-656.

Definition of voltammetry: the potential of the working (WKG) electrode is controlled with respect to the reference (REF) electrode. The analyte is oxidized or reduced at the WKG electrode surface. The resulting current is plotted vs applied potential in a voltammogram.

Voltammetry is useful both for qualitative analysis (identification of analyte) and quantitative analysis (measurement of concentration). Unlike potentiometry, voltammetry measures concentration, not activity. Finally, voltammetry is virtually the only instrumental method that is truly specific for the oxidation state of the analyte (e.g., how much Fe(II) and how much Fe(III) are present).

We will focus first on general principles of voltammetry, and then on two widely used methods: steady state voltammetry and cyclic voltammetry.

A. The Voltammetry Experiment.

The electrochemical cell consists of a solution, 3 electrodes, and a means of passing nitrogen (or argon) through the solution or over the solution. The solution, also known as the electrolyte, contains the analyte and a large concentration of inert ions (known as the supporting electrolyte). The 3 electrodes are called the working (WKG), reference (REF) and counter (CTR) electrodes.

WKG - the electrode at which the analyte is oxidized or reduced, generating a current.

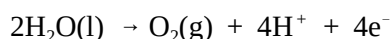
REF - the electrode that provides a defined half-cell potential.

CTR - the electrode that completes the current path through the cell.

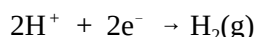
The cell is connected to a potentiostat. The potentiostat performs 3 functions:

- (a) it controls the applied potential, which is potential difference between the WKG and REF electrode (the applied potential controls what half reactions occur at the WKG electrode);
- (b) it passes current between the WKG and CTR electrodes without passing current through the REF electrode (which would change its potential if current did pass through it);
- (c) it converts the cell current to a voltage for recording devices.

Typical WKG electrodes are solid metals (Pt, Au), a liquid metal (Hg), and a quasi-metal (carbon as graphite or doped diamond). At sufficiently positive potentials, both the electrolyte (especially the water) and the electrode oxidize. Water oxidation generates $O_2(g)$:



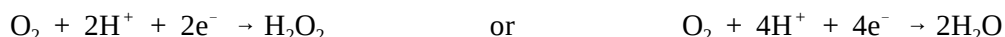
The electrodes grow surface oxides (Pt, Au, C(gr)) or they dissolve ($2Hg \rightarrow Hg_2^{2+}$). At sufficiently negative potentials, the water reduces to $H_2(g)$:



The range of potentials over which none of these half-reactions occur is the electrochemical window. The electrochemical window varies with the electrode and the pH of the electrolyte.

Typical REF electrodes are the SCE and the Ag/AgCl. Typical CTR electrodes are Pt or Au or graphite.

Oxygen removal - Dissolved oxygen is reducible to H_2O_2 or water:



This generates an annoying background current. Dissolved O_2 is removed by sparging - bubbling an inert gas (N_2 or Ar) through the electrolyte for at least 10 minutes.

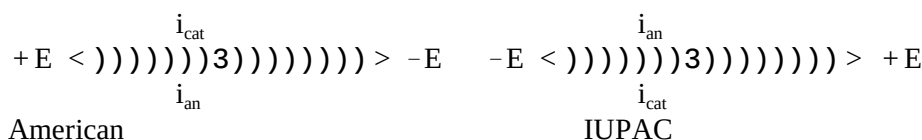
B. Voltammogram Convention.

A voltammogram is a plot of the current flowing through the WKG electrode as a function of the applied potential ($E = E_{\text{WKG}} - E_{\text{REF}}$). Define cathodic current as current corresponding to a reduction (e.g., $\text{Fe}^{3+} + e^- \rightarrow \text{Fe}^{2+}$), and anodic current as current corresponding to an oxidation (e.g., $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + e^-$).

There are 2 conventions in the literature:

American - plot cathodic current positive, plot negative potentials decreasing to the right.

IUPAC - plot anodic currents positive, plot positive potentials increasing to the right.



The slightly illogical American convention arose for historical reasons. SH&N use the American convention in all voltammograms. Keep in mind that reductions will appear as positive current, oxidations as negative current. Incidentally, IUPAC stands for International Union of Pure & Applied Chemistry.

C. Mass Transfer (how the analyte gets to the electrode).

1. Convection - bulk flow of the solution by stirring or pumping.
2. Diffusion - movement of a solute due to random motion of molecules. Diffusion causes solutes to move down a concentration gradient i.e. from high conc. to low conc., and is always present.
3. Ion migration - movement of ions from anode to cathode or visa-versa to carry current. Movement of an analyte ion by this mode is undesirable, so it is suppressed by the addition of a supporting electrolyte - a 100-fold excess of some inert salt, strong acid or strong base. This also lowers the cell resistance, which is desirable in voltammetry.

Review

Definitions: Voltammetry, WKG, REF & CTR electrodes; supporting electrolyte; cathodic & anodic current; American convention in voltammograms; electrochemical window; sparging.

Concepts: What are the 3 functions of a potentiostat? Why and how is oxygen removed from the electrolyte? What are the three forms of mass transport? What are the 2 roles of supporting electrolyte?

XX. STEADY STATE VOLTAMMETRY.

A class of voltammetric methods in which the electrolyte moves relative to the WKG electrode and the resulting currents are independent of time (steady-state). Widely used in electrochemical detectors for liquid chromatography.

Assumptions:

1. The analyte is part of a half-rxn:

$$\text{O} + n e^- \rightleftharpoons \text{R}$$
with formal potential E^0

The analyte is either O (oxidized form) or R (reduced form). We will assume O is the analyte for this lecture. (Ex. $\text{Fe}^{3+} + \text{e}^- \rightarrow \text{Fe}^{2+}$; Fe^{3+} is the analyte; $\text{Pb}^{2+} + 2\text{e}^- \rightarrow \text{Pb(s)}$; Pb^{2+} is the analyte)

2. The solution is stirred; convection & diffusion control the mass transfer of O and R to and from the electrode. Convection keeps the concentrations of O and R constant in the bulk electrolyte. Concentrations of O and R are different at the electrode surface, depending on the applied potential.
3. The electrode area is small and the electrolyte volume is large, so bulk concentrations of O and R do not change during the experiment.
4. The Nernst equation is obeyed at the WKG electrode surface (reversible behavior) due to fast kinetics of e^- transfer.

$$E_{\text{wkg}} = E^{\circ'} - (0.059/n) \cdot \log([R]_s/[O]_s) \quad (\text{subscript s means surface concentrations of R and O})$$

These assumptions lead to the characteristic steady-state voltammogram (sketch). Note that the current is zero at potentials positive of $E^{\circ'}$, and the current rises to a limiting value (limiting current) at potentials negative of $E^{\circ'}$. The current is cathodic because a reduction is taking place at the electrode ($\text{O} + \text{ne}^- \rightarrow \text{R}$). The limiting current is proportional to conc. of analyte (quantification).

$$i_l = mC \quad \text{where } m = nFA(r_{\text{mt}})$$

n is the # moles of electrons transferred per mole of analyte in the half-reaction, F is Faraday's constant (96485 coul/mol), A is the electrode area, and r_{mt} is the rate of mass transfer (the rate increases as convection increases). m is the sensitivity and is determined by any of the 3 calibration methods.

At $i = i_l/2$, then $E = E_{1/2}$, the half-wave potential. The half-wave potential is very close to the formal potential $E^{\circ'}$ (identification).

Ex. Sketch a steady state voltammogram for a solution containing Fe^{3+} . The half reaction is:
 $\text{Fe}^{3+} + \text{e}^- \rightarrow \text{Fe}^{2+} \quad E^{\circ} = +0.50 \text{ V vs SCE}$

The equation describing the voltammogram is:

$$E_{\text{wkg}} = E_{1/2} - (2.3RT/nF) \cdot \log(i/(i_l - i))$$

A plot of E_{wkg} vs $\log(i/(i_l - i))$ is linear with a slope of $-(2.3RT/nF)$ (yields n) and x-intercept $E_{1/2}$. Recall that at 25°C , $2.3RT/nF = (0.05916 \text{ V})/n$.

Review

Definitions: half-wave potential; limiting current.

Concepts: What are the assumptions of steady state voltammetry? Sketch a steady-state voltammogram (American convention) given the analyte, the half-reaction and the formal potential. How does the SSV provide information about the identity, concentration, and oxidation state of the analyte?

Calculations: Calibration curve and standard addition problems. Calculation of formal potential and # of electrons in the half-reaction from current-potential data.

XXI. LINEAR SCAN AND CYCLIC VOLTAMMETRY.

Reading: Harris, ch. 17, pp.394-396.

Assumptions: Same 4 assumptions as with SSV except:

2. The solution is not stirred - no convection.

The electrode and the electrolyte are both stationary; the only form of mass transfer is diffusion.

The WKG electrode potential is scanned linearly with time from E_i to E_s (E_s is the switching potential) (LSV) and back to E_i (CV). The magnitude of the scan rate v is constant; a typical value is 0.1 V/s. Usually E_i is selected to be in a region of zero current (potential well positive of $E^{0'}$) and the scan direction is selected to take the potential into the reduction of **O**. E_s is selected to be well negative of $E^{0'}$. The voltammogram shape is complex because the reactant **O** is consumed near the electrode and the product **R** is generated near the electrode. On the forward scan, the current exhibits a peak about 30/n mV beyond $E^{0'}$. The current peak is proportional to the concentration of the analyte (quantitative analysis).

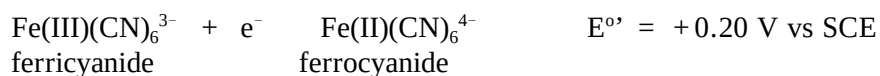
$$i_p = mC \quad \text{where } m \text{ is the sensitivity}$$

The sensitivity m is proportional to the square root of the scan rate ($\sqrt{v}$). Doubling the scan rate causes the peak current to increase by a factor of $\sqrt{2} = 1.4$. When the scan is reversed beyond the peak (CV), the product **R** is re-oxidized back to the reactant **O**, and a second peak of the opposite sign is observed at about 30 mV/n beyond $E^{0'}$. A useful means of locating $E^{0'}$ is given by:

$$E^{0'} = (E_p^c + E_p^a)/2 \quad \text{where } E_p^c \text{ is the cathodic peak potential and } E_p^a \text{ is the anodic peak potential}$$

Because CV is a fast experiment (less than a minute for a complete scan), it is an excellent survey method for rapidly exploring the electrochemical behavior of a new compound, or rapidly locating the half-wave potentials of analytes (qual. analysis). It is less commonly used for quant. work; hydrodynamic methods are superior in accuracy and precision.

Ex. An electrolyte contains ferricyanide ($\text{Fe(III)(CN)}_6^{3-}$). Ferricyanide is part of the following half reaction in 0.1 M KCl:



Sketch the steady state voltammogram and the cyclic voltammogram. Indicate how the concentration of the ferricyanide could be obtained from each voltammogram. Indicate how the formal potential could be obtained from each voltammogram. (this problem will be answered in class)

Ex. A gold bead electrode was used to measure ferricyanide concentration by cyclic voltammetry. A 1.0 mM solution yielded a peak current of 205 μA (microamperes) at a scan rate of 0.1 V/s. What would be the peak current for the 1.0 mM ferricyanide solution if the scan rate was 0.5 V/s?

Since i_p is proportional to m which is proportional to $\sqrt{v}$, set up a ratio.

$$i_p/\sqrt{v} = \text{constant. So, } 205/\sqrt{0.1} = i_p/\sqrt{0.5} \quad \text{and } i_p = 205\sqrt{0.5}/\sqrt{0.1} = 458 \mu\text{A}$$

Review

Concepts: What assumption for cyclic voltammetry is different from the corresponding assumption for steady state voltammetry? Define the potential waveform for a CV (sketch). Sketch a CV given the analyte, its half-reaction and its formal potential; identify the initial and switching potentials.

Calculations: standard addition and calibration curve problems; calculation of the formal potential from the peak potentials; peak current vs scan rate.

XXIII. ANODIC STRIPPING VOLTAMMETRY.

Reading: Harris, ch. 17, 393-394.
SH&N, ch. 25, pp. 666-668.

Stripping voltammetry has the lowest L.O.D. of the electrochemical methods (down to 10^{-10} M). It is capable of analyzing more than one substance at a time. It is based on 2 steps:

- (a) Preconcentration - the analyte is deposited on or into the WKG electrode by reduction or oxidation at the deposition potential E_{dep} . The solution is stirred to increase mass transfer of analyte to the electrode.
- (b) Stripping - the deposited film is stripped off or out of the WKG electrode during a potential scan that reverses the deposition reaction. The solution may be stationary during this step. The stripping voltammogram yields qual. and quant. information.

A. Anodic Stripping Voltammetry (ASV).

The deposition step is a reduction; the stripping current is anodic. Principally used for metal cations that can be reduced at Hg electrodes.



To choose E_{dep} , look at the voltammogram of the analyte in the sample (sketch). E_{dep} should be on the limiting current plateau, where every analyte ion reaching the electrode surface is reduced. Since more than one metal can be deposited and analyzed on the stripping scan, ASV is capable of multi-element analysis. Deposition more than one metal can lead to intermetallic interferences - metals forming alloys that do not strip as separate metals.

Stripping can be done by a linear scan, where E scans positive from E_{dep} . (Sketch of stripping voltammogram) More complex potential-time waveforms are often used because they offer improved LODs. Current peaks correspond to the removal of deposited metals at their characteristic potentials. Peak position gives quant. analysis ($E_p \approx E^0$); peak height or peak area gives qual. analysis ($i_p = mC$).

B. Working Electrodes for ASV.

Typically Hg electrodes are used. Discussion will be limited to the mercury film electrode (MFE). The MFE is formed by adding Hg^{2+} (ca. 10^{-4} M) to the sample and reducing Hg^{2+} to Hg simultaneously with reduction of the metal ion; the substrate is a graphite WKG electrode. Because the volume of the Hg film is very small, metals are preconcentrated to a high degree (but intermetallic interferences increase). The stripping peaks are sharp because all of the metal is stripped out in a few seconds; this leads to high sensitivity and better resolution of peaks for different analytes (voltammogram sketch).

C. The Stripping Voltammetry Experiment.

Precision for this method tends to be worse than for other voltammetry methods because of the deposition step. The cell contains 3 electrodes and some means of controlled stirring. A typical procedure is:

- (1) Sparge the electrolyte to remove dissolved oxygen. Apply E_{dep} for a fixed time (30 s to 30 min) while stirring at a reproducible rate. Longer deposition times yield lower LODs. The stirring may be turned off for the last 30 s if the stripping step is to be done in quiet solution.
- (2) Perform the stripping scan; scan in the positive direction from E_{dep} .
- (3) Condition the WKG electrode for the next scan i.e. dispense new Hg drop or strip off old Hg film.

Automation of the procedure by computers greatly improves precision. ASV is particularly useful for analysis of the “heavy metals” ions (e.g., Cu^{2+} , Zn^{2+} , Cd^{2+} , In^{3+} , Pb^{2+} , Tl^{+}) in aqueous samples. Standard addition is the preferred calibration method.

Review

Definitions: stripping voltammetry, preconcentration.

Concepts: Why is stripping voltammetry so sensitive? Describe the stripping experiment (deposition potential, preconcentration, stripping, half-reactions). Sketch a stripping voltammogram. Describe a MFE.

Calculations: Standard addition and calibration curve problems.

XXIV. COULOMETRIC METHODS.

Reading:

SH&N, ch. 24, pp. 627-632.

A General Characteristics.

The characteristics of coulometric methods are unique:

1. All coulometric methods are based on the exhaustive electrolysis of the analyte; greater than 99% of the analyte in the sample is converted from one oxidation state to another.
2. Coulometric methods are strictly quantitative; the identity, oxidation state and formal potential of the analyte are known.
3. Coulometry is an absolute method; no calibration is required. Quantification is based on Faraday's law:

$$Q/nF = \# \text{ moles of analyte} = VC$$

where Q = # of coul required for exhaustive electrolysis; n = # of mole e^- per mole of analyte (look at the half-reaction); F = 96485 coul e^- /mole e^- ; V = volume of electrolyte; C = molar conc. High accuracy and precision are possible, on the order of $\pm 0.1\%$ or better.

Q , the number of coulombs passed, is obtained by electronic integration of the current over time:

$$Q = \int i(t) dt \quad \text{coul} = \text{amperes} \cdot \text{s}$$



During exhaustive electrolysis of a cell containing I^- in 10.00 mL of solution, 15.00 coul of charge was required to convert all of the I^- to I_2 . Calculate the # of moles of I^- and the conc. of I^- .

Start with the half-reaction: $I_2 + 2e^- \rightarrow 2I^-$. This half-reaction is being run in the reverse direction. Every 2 moles of iodide generates 2 moles of electrons, so $n = 1 \text{ mol } e^-/\text{mol } I^-$.

$$Q/nF = 15.00 \text{ coul}/[(1)(96485 \text{ coul/mol})] = 1.555 \times 10^{-4} \text{ mol } I^-$$

$$(1.555 \times 10^{-4} \text{ mol})/(0.01000 \text{ L}) = 1.555 \times 10^{-2} \text{ M}$$

B. Controlled Potential Coulometry (CPC).

Basic experiment: The potential of a WKG electrode is held at a value needed to convert the analyte quantitatively to another oxidation state. The conc. of the analyte and the current decay exponentially. At a predetermined point, the electrolysis is stopped and the integrated current is used to calculate the amount of analyte present.

Assume the analyte is O. Then E_{app} must be at least $180/n \text{ mV}$ more negative than $E^{0'}$ to convert 99.9% of O to R (this rule of thumb can be derived from the Nernst equation).

In CPC, a 3-electrode cell is used, with the CTR and REF electrodes isolated by frits or other liquid junctions. To speed up the electrolysis, the WKG electrode area is large, the solution volume is relatively small, and the solution is stirred as vigorously as possible. The conc. of the analyte and the resulting current decay exponentially (diagrams):

$$i = i_0 \cdot e^{-t/\tau} \quad \text{or} \quad \ln(i) = \ln(i_0) - t/\tau$$

where i_0 is the initial current and τ is the electrolysis time constant (units s). τ is proportional to the volume of solution and inversely proportional to the stirring rate and the electrode area. It is desirable to make the electrolysis as short as possible, which means τ should be as small as possible. Hence, the WKG electrode area should be as large as possible, the solution volume as small as possible, and the stirring rate as fast as possible.

The charge passed (measured with an electronic integrator) increases exponentially to a limiting value (diagram):

$$Q = Q_{total} (1 - e^{-t/\tau})$$

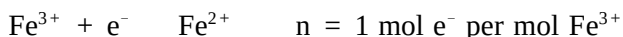
The current is integrated until the current has decayed to 0.1% of its initial value ($i/i_0 = 0.001$). Then:

$$Q = 0.999 \cdot Q_{\text{total}} \text{ when } e^{-t/\tau} = 0.001; -t/\tau = \ln(0.001) = -6.91; t = 6.91\tau$$

The accuracy of this analysis is 0.1%.

Question: If a minimum accuracy of 1% is needed, then what is the current level at the end of the electrolysis, and how long will the electrolysis be in terms of electrolysis time constants? Answers: 1% of i_0 , $t = 4.61\tau$ ($\ln(0.01) = -4.61$)

Ex. A 2.00 mM solution of Fe^{3+} is reduced by CPC at a Pt WKG electrode of 4.0 cm^2 area in a cell whose volume is 20.0 ml. If the electrolysis time constant τ is 333 s, what is Q_{total} and the time for 99% and 99.9% conversion to Fe^{2+} ?



$$Q_{\text{total}} = nFN = (1)(96485 \text{ coul/mol})(2.00 \times 10^{-3} \text{ M})(0.0200 \text{ L}) = 3.86 \text{ coul}$$

$$\text{Time for 99\% electrolysis} = 4.61\tau = (4.61)(333 \text{ s}) = 1540 \text{ s} = 25.6 \text{ min}$$

$$\text{Time for 99.9\% electrolysis} = 6.91\tau = (6.91)(333 \text{ s}) = 2300 \text{ s} = 38.4 \text{ min}$$

Review

Definitions: exhaustive electrolysis; controlled potential coulometry; electrolysis time constant.

Concepts: What are the general characteristics of coulometric methods? Why is coulometry an absolute method? Given the formal potentials of solution species, choose an appropriate applied potential for exhaustive electrolysis of the analyte. What factors lead to shorter electrolysis times? What is the time dependence of the current in CPC? How does one know when the electrolysis can be stopped?

Calculations: Calculate concentration or amount from Faraday's law. Calculate the electrolysis time constant and time required for 99.9% (or some other criteria) electrolysis of the analyte.

XXV. FUNDAMENTALS OF CHROMATOGRAPHY.

Reading:

SH&N, ch. 26, pp. 674-696.

A. Chromatography Basics.

The basic definition of chromatography is the separation of substances based on their distribution between a mobile phase and a stationary phase. A modular diagram for a chromatograph is:

mobile phase → (pump) → sample injection → column (inside programmable oven) → detector → readout

Inside the column, the sample exchanges between the mobile and the stationary phase. Analytes that interact more strongly with the stationary phase and more weakly with the mobile phase tend to take longer to pass through the column. Types of interactions include dispersive (London forces), polar (dipole-dipole and dipole-induced dipole), ionic (coulombic attraction and repulsion) and size-exclusion. The detector responds to a property of the analyte (e.g., absorbance, fluorescence, electrochemical reaction, mass

spectrum) or of the mobile phase (e.g., index of refraction, thermal conductivity). The chromatogram is a plot of detector response vs time (sketch). Ideally, each peak corresponds to an single analyte. The time of elution of the peak provides evidence for the identity of the analyte (qual. analysis) and the height or area of the peak can be related to the concentration of the analyte (quant. analysis).

Chromatography is subdivided according to mobile phase first and stationary phase second:

Mobile phases: gas (G), liquid (L) or supercritical fluid (SF).

Stationary phases: solid (S) or liquid (L) (this is often omitted).

Common acronyms include GC or GPC (gas chromatography or gas phase chromatography), LC (liquid chromatography), HPLC (high performance LC), and SFC (supercritical fluid chromatography). Many other variations in names and modes of operation are in the literature.

We will focus on GC and LC. In both of these methods, a small volume of sample is placed at the head of the column and eluted with fresh mobile phase (eluant). The column is the most critical piece of hardware. Packed columns contain powder particles of roughly uniform size. Either the powder surface is the stationary phase, or the stationary phase (a nonvolatile, insoluble liquid or solid) is coated on the powder. Capillary columns are open tubes with the stationary phase attached to the walls of the capillary.

B. Chromatography Theory.

Chromatography theory falls into two categories, plate theory and rate theory (a.k.a. kinetic theory). Neither theory is correct, but they provide useful tools for understanding how to separate analytes in a mixture. We start with some observable quantities in the apparatus and in the chromatogram.

Apparatus:

L = length of the column (cm)

u = average linear rate of movement of the mobile phase (cm/s)

t_M = dead time = time for non-retained species to move through the column (s)

Hence: $u = L/t_M$

(i) Chromatogram with an isolated peak: The horizontal axis can be time or volume of the mobile phase. We will use time as the horizontal axis.

t_R = retention time = time from sample injection to the peak (s)

t_R' = adjusted retention time = $t_R - t_M$

W = width of the base of the triangle which approximates the peak (diagram) (s)

(ii) Chromatogram with two closely spaced peaks A and B, with B eluting after A:

$$R = \text{resolution} = (t_R(B) - t_R(A)) / (W_A/2 + W_B/2)$$

Peaks are said to be completely resolved if $R \geq 1$.

The main objective in chromatography is to maximize R while minimizing t_R . There are 2 strategies for maximizing R: (i) increase the difference in retention times, and (ii) decrease peak widths

1. Plate Theory.

The column is divided into imaginary theoretical plates. Within each plate, the analyte is assumed to be equilibrated between the stationary and mobile phases. The equilibrium constant (partition coefficient, distribution coefficient) K is the ratio of concentrations C_S/C_M , where C_S is the conc. of the analyte in the stationary phase and C_M is the conc. of the analyte in the mobile phase.

Define $k' = \text{capacity factor}$. For each analyte:

$$k' = t_R'/t_M = (t_R - t_M)/t_M$$

For various reasons, separations are optimum for capacity factors between 1 and 5.

For two analytes A and B, a given column has a selectivity factor $\alpha = K_B/K_A$ which reflects the relative interactions of A and B with the stationary phase of the column. By definition, B is the more strongly retained species ($K_B > K_A$), so α is always greater than 1. Again, one can derive:

$$\alpha = k_B'/k_A' = t_R'(B)/t_R'(A)$$

2. Kinetic Theory.

A plug of analyte traveling through a column will spread out along the column axis (band broadening - diagram) for several reasons: (a) multipath - in packed columns, different molecules follow different paths of varying lengths; (b) diffusion - molecules in the mobile phase will diffuse from high conc. to low conc.; (c) mass transfer - different molecules will spend different lengths of time in the stationary phase, where they do not move along the column. The plug broadens into a Gaussian distribution (bell curve) of concentration with distance x along the column:

$$C(x) \propto \exp[-(x - x_i)^2/(2\sigma^2)]/(\sigma(2\pi)^{1/2})$$

σ^2 is the variance which characterizes the width of the distribution; σ is the standard deviation. The width of the distribution at 0.607 of the height is 2σ . When the plug passes the detector, the chromatogram displayed is a Gaussian peak in time. In general, the width of the Gaussian increases with the retention time t_R .

Column performance is characterized by two related quantities: plate height H (units of distance) and the number of theoretical plates N (unitless) (do not confuse this N with the # of repetitive measurements).

The theoretical plate is an artificial construct from Plate Theory.

$$N = L/H$$

The plate height is defined as the variance of the plug per unit length of the column:

$$H = \sigma^2/L \text{ (units of cm)}$$

Then $N = L^2/\sigma^2$. Hence, a large value of N (a small value of H) implies a narrow peak; the chance that the peak is resolved from adjacent peaks is improved. N is usually estimated by drawing a triangle whose sides are tangent to the chromatogram peak; the base of the triangle is W . Then:

$$N = 16(t_R/W)^2 \text{ (both } t_R \text{ and } W \text{ have units of time)}$$

Ex. A gas chromatogram of a pesticide using a 20.0 cm column yielded a peak with a retention time of 13.3 minutes and a width of 0.12 minutes. Calculate N and H .

$$N = 16(13.3/0.12)^2 = 2.0 \times 10^5 \text{ (unitless); } H = L/N = (20.0 \text{ cm})/2.0 \times 10^5 = 1.0 \times 10^{-4} \text{ cm}$$

An alternative method for approximating N is based on the peak width at half-maximum, $W_{1/2}$. Then:

$$N = 5.54(t_R/W_{1/2})^2$$

Theory and experiment show that H depends on the flow rate u of the mobile phase:

$$H = A + B/u + Cu \quad (\text{the } \underline{\text{van Deemter equation}})$$

A, B, and C are theoretical constants related to the 3 band-broadening processes mentioned above (multipath, diffusion, mass transfer). A van Deemter plot of H vs u reveals that H has a minimum value (and hence N has a maximum value) at one flow rate. Optimum flow rates are much higher for GC compared to LC. Theory and experiment also indicate that H decreases as (a) the diameter of the column decreases and (b) the particle size in packed columns decreases. Hence, there has been a trend towards smaller columns in both types of chromatography.

Two ideas are worth mentioning. The first is that resolution is proportional to the square root of the number of theoretical plates:

$$R = k\sqrt{N} \quad (\text{where } k \text{ is a proportionality constant, not the capacity factor})$$

$$\text{Hence } R = k\sqrt{L} \quad (\text{since } N \propto L)$$

Thus, to double the resolution, the column length must be increased by a factor of 4. If the flow rate is constant, then the retention time also increases by a factor of 4 (since $t_R \propto L$). Since time = money (Einstein's second famous equation), using a longer column is not usually an economic option.

The second idea is the general elution problem. In brief, a complex mixture with many components is difficult to separate optimally (k' between 1 and 5, no overlapping peaks) under a single set of conditions (e.g., column length, flow rate, mobile phase, stationary phase, temperature). The solution is to vary one of the conditions during the separation process. For gas chromatograph, the temperature is increased (temperature ramping), while for liquid chromatography, the composition of the mobile phase is changed (gradient elution). Then, analytes with widely varying partition coefficients can be eluted over a relatively short time with minimal loss of resolution.

Review

Definitions: Stationary & mobile phases; packed & capillary columns; theoretical plates; retention time and dead time; capacity factor; selectivity ratio; resolution; internal standard.

Concepts: What is the main objective in chromatography? What is the van Deemter equation and what parameters can be optimized using a van Deemter plot? What are the 3 factors which cause band broadening in chromatography? What is the general elution problem, and what is done about it?

Calculations: Calculate k' , H, N, R, and α from relevant data (t_R , t_M , W, L). Calculate the effect of changes of L on R, N, and t_R .

C. Qualitative and Quantitative Analysis. (this section won't be covered in lecture)

Qual. Analysis: Many of the common detectors do not provide information on the identity of the analyte. Consequently, the presence of a peak at the same retention time as the retention time of a standard is not sufficient to prove the presence of the compound. However, the absence of the peak is a valid indicator that the compound is not present (or is at very low conc.).

Quant. Analysis: Assumptions: (a) you know the identity of the analyte and (b) the detector signal is linear with concentration of the analyte ($S = mC + S_{bl}$). The sensitivity m is usually different for different analytes. S can be the peak height or the peak area; the peak area is more reliable, and is often available from electronic integrators. Possible methods for quantifying the analyte are:

1. Calibration curve. (You remember this, don't you?)
2. Standard addition. (ditto)
3. Internal standard - (review this concept as defined in atomic emission spectroscopy). The internal standard should elute at nearly the same time as the analyte, but be cleanly separated from it and every other peak. Internal standards improve precision by reducing error due to varying injection volume. They are used in both calibration curve and standard addition calibration methods.

XXVI. GAS CHROMATOGRAPHY.

Reading:

SH&N, ch. 27, pp. 701-717.

The modular diagram for a chromatograph:

carrier gas supply → (pressure/flow regulator) → *sample injection* → *column* → *detector* → readout
(mobile phase)

Everything in *italics* is in a temperature controlled environment. The sample must be a vapor within this environment.

We will focus on GLC (henceforth just GC), the most wide-spread method. Modules to consider include the mobile phase, sample injection, the oven, columns and detectors.

A. The Mobile Phase (Carrier Gas).

Requirements: high purity, compatibility with the detector, controlled flow rate. Filters and traps are often placed between the tank of gas and the GC unit to remove particles, oil drops, O_2 and H_2O . The identity of the mobile phase does not affect the partitioning of the analyte into the stationary phase. The usual choices for carrier gas are He , H_2 , N_2 , or Ar . Van Deemter plots for different gases show that H_2 and He offer minimum H (maximum N) for higher flow rates than the other gases → shorter analysis times. Typical linear velocities are 20 - 80 cm/s. Flow rate can be controlled by simply regulating the pressure of the carrier gas at the column head, but the flow rate will vary with column temperature (the back pressure will change).

B. Sample Injection Systems.

Samples may be gases or liquids. To avoid column overload (injecting more sample than can dissolve in the stationary phase), sample liquid volumes of 0.1 to 10 μL are used for packed columns and 0.01 μL for capillary columns. Sample injections in the 0.1 to 10 μL range are made through a silicone rubber septum using precision syringes. The injection chamber must be hot - at least $50^\circ C$ above the boiling point of the highest boiling component - to flash-evaporate the liquid. A typical injector temperature is $300^\circ C$. For capillary columns, use a split/splitless sample injector (diagram). In the splitless mode (useful for trace

analysis), all of the sample goes into the column. In the split mode (preferred), flow of carrier gas out an exit port carries away most of the vaporized sample. Roughly 1% of the injected sample enters the column.

Too much sample injected (column overload) leads to a chromatogram exhibiting peak fronting (diagram).

C. The Oven.

Requirements: (a) temperature control to $\pm 0.2^\circ\text{C}$, (b) temperature range from ambient or below to 300°C or higher, (c) temperature settings to the nearest 1°C , and (d) temperature uniformity throughout the oven (a fan are used).

For simple separations and column evaluation, usually the temperature is held constant (isothermal).

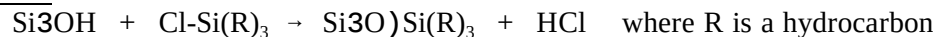
Temperature programming allows the column temperature to increase at a preset rate during the analysis of a sample. This powerful method allows the analysis of samples with widely varying retention times with improvement in resolution and sensitivity. It is especially useful for complex mixtures because retention times (and k') decrease as the temperature increases (sketch). The minimum programming capabilities are (a) hold at T_1 for time t_1 , (b) ramp the temp. to T_2 at rates of 1 to $20^\circ\text{C}/\text{min}$, (c) hold at T_2 for time t_2 , (d) return rapidly to T_1 and equilibrate the column in minimum time (diagram).

D. Columns. We will consider the support for the stationary phase and the stationary phase itself.

The support.

1. Packed columns are metal or glass tubes packed with diatomaceous earth particles. The particles are coated with the stationary phase. Ideally the particles should be as small as possible (higher N) and have a very high surface area (higher loading capacity - the maximum amount of material that can be injected without losing linear partition equilibria). However packing uniformity and pressure drop problems prevent the use of very small particles; the balance is struck at 100-300 μm particles with a surface area of 1-4 m^2/g . Typical column lengths are 1-6 m; diameters are 2-4 mm; N values are 10^3 - 10^4 plates.

Active sites are exposed SiOH groups (silanols) of the particles; they cause peak tailing (diagram), especially for acids, alcohols and amines due to strong hydrogen bonding of these analytes at the active sites. Active sites are sometimes passivated after column coating by silanization:



2. Capillary columns are now made from fused silica (SiO_2) with a polyimide coating (which prevents the silica from snapping when the column is coiled). The loading capacity is low, but resolution is high. Column lengths are 10-100 m, the inside diameter is 0.1-0.5 mm, and $N \approx 10^4$ - 10^5 plates. Capillary columns provide better resolution because (a) they are 5 - 10 times longer than packed columns, and (b) they don't have band broadening due to the multipath process. Van Deemter plots show that faster gas velocities can be used on capillary columns, leading to shorter analysis times. The combination of superior efficiency (higher N) and shorter separation time has caused these columns to dominate modern GC.

The stationary phase:

$$\text{R} \quad \text{R} \quad \text{R}$$
$$\text{R})\text{Si}(\text{O})[\text{Si}(\text{O})]_n \text{Si}(\text{R})(\text{R}) \quad \text{R} = \text{CH}_3, \text{phenyl}, \text{CH}_2\text{CH}_2\text{CF}_3, \text{CH}_2\text{CH}(\text{N})$$
$$\text{R} \quad \text{R} \quad \text{R}$$

CF₃ or CN: very polar, little polarizability, weak hydrogen bonding

E. Detectors.

3. Electron capture detectors (ECD) (sketch) are selective for electronegative groups, mainly halides but also peroxides, quinones and nitro groups. ECD's are based on the emission of electrons into the carrier gas by a beta emitter (^{63}Ni or ^3H). The preferred carrier gases are nitrogen or argon. A current is produced between 2 electrodes several hundred volts apart in potential. The electronegative analyte molecules capture the electrons irreversibly and reduce the number of electrons collected by the electrodes; the signal is a decrease in current. ECD's are very sensitive

with a very low LOD and a wide dynamic range. ECD's are the detector of choice for PCB's and chlorinated pesticides. ECD's are sensitive to impurities (O_2) in the carrier gas.

Review

Definitions: Column overload; peak fronting; peak tailing; silanization; bleeding.

Concepts: Sketch a gas chromatograph, and choose a mobile phase gas, type of column, and detector; justify your choices of mobile phase and detector. Describe how a split/splitless injector is used with a capillary column. Describe a typical temperature program. What are the advantages of capillary columns over packed columns? What determines the order of elution for similar analytes? Sketch and describe the operation, sensitivity, dynamic range and selectivity of the three major types of GC detectors: TCD, FID, ECD.

XXVII. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY.

Reading:

SH&N, ch. 28, pp. 725-750.

One of the most widely used forms of instrumental analysis. HPLC can handle nonvolatile samples and thermally sensitive samples which cannot be analyzed with a GC. The liquid mobile phase adds an extra dimension of control to chromatography since the analyte interacts with the mobile phase as well as with the stationary phase. The module diagram for LC is:

Mobile phase reservoirs → (mixing valve) → pump → injection valve → column → detector → signal processing

The mixing valve and pump are programmable by a computer.

A. Types of LC.

Classifications of LC are actually based on the types of interactions between the analyte and the stationary and mobile phases.

1. Normal phase chromatography uses a polar stationary phase and a nonpolar mobile phase. Typical stationary phases are silica (SiO_2) and alumina (Al_2O_3).
2. Reverse phase chromatography uses a nonpolar stationary phase and a polar mobile phase. The silica support is silanized with C_4 , C_8 or C_{18} silanes to create a nonpolar stationary phase. We will focus on this type of LC.

(The next two types will not be covered in lecture, but are left here for completeness).

3. Ion exchange chromatography uses an ionic stationary phase to separate ionic analytes. The stationary phase can be a polymer with anionic (e.g. $-SO_3^-$ or $-COO^-$) or cationic (e.g. $-NR_3^+$) sites.
4. Size exclusion chromatography uses a polymeric stationary phase with well-defined pores. The analyte molecules that partly penetrate the pores are retained longer in the stationary phase. Molecules that are too large will all elute with the dead volume. Used for analysis of polymers and proteins.

B. The Mobile Phase

Requirements: (a) high purity, (b) freedom from particles (use on-line filter), (c) "degassed" to remove dissolved O₂ and N₂; the latter gases can cause bubble formation (a disaster!) during pressure changes in the column. Either the mobile phase is exposed to a partial vacuum or it is saturated with He (which has low solubility in liquids).

Choice of solvent or solvent mixture affects separation enormously and may depend on the type of detector used e.g. transparency requirement for a UV/VIS detector. For reverse phase LC, typical solvents are water, alcohols, acetonitrile, and hexane, all of which are UV-VIS transparent. Often the pH is controlled with added acid or buffers.

Elution with a single solvent or a constant solvent mixture is called isocratic elution. Gradient elutions are done by mixing 2 or more solvents and changing the composition of the mixture while the sample is passing through the column. Gradient elutions (like temperature programming in GC) allow analytes with widely differing retention times to be separated quickly with improved resolution and sensitivity. Gradient elutions usually involve a change in mobile phase polarity. To achieve a gradient elution, a programmed mixing valve controls the percentage of solvent from each reservoir that goes to the pump.

C. The Pump System.

Requirements: (a) operating pressures up to 6,000 psi, (b) precise and accurate flow rates of ca. ml/min., and (c) pulse-free.

Reciprocating pumps are based on a reciprocating piston with check valves. Disadvantage - flow output is pulsed requiring both (a) a pulse damper and (b) a multiple piston design. Advantages - constant flow rate, works well with gradients.

D. Sample Injection.

The rotary injection valve allows injection of precise volumes at high pressure. It has two positions (sketch). In the load position, mobile phase is sent to the column directly. A sample loop is filled by injection via a port and a waste outlet. Injection volumes are defined by the sample loop and are typically 5 - 100 µL. In the inject position, the sample loop is placed in-line with the mobile phase. The sample is sent towards the column.

E. The Column.

The support.

Because of the high pressures required, the column is made of stainless steel. Greater chemical inertness can be obtained with glass-lined columns. The columns are packed with (usually) porous silica particles of 3-10 µm, the optimum size for maximum resolution and minimum pressure drop across the column. Column lengths of 3-30 cm and diameters of 4.6 mm are typical. Typically the column is operated at room temperature.

The stationary phase.

Stationary phases are most commonly nonpolar silanes with chain lengths of 4, 8 or 18 carbons which are bonded to the silica particles. Normally the column is used at room temperature, but thermostating the

column is desirable. Bonded silanes tend to be hydrolyzed at alkaline pH's, so the mobile phase pH is kept neutral to acidic.

The order of elution of analytes is strongly dependent on the mobile phase and on the chemical interactions between the analytes and the stationary phase. In reverse-phase chromatography, the most polar analytes tend to elute first because these have the weakest interaction with the nonpolar stationary phase. For analytes of similar polarity, the analytes with the lowest molecular weights tend to elute first. There are a number of procedures for finding and optimizing a solvent combination; we will not cover them here. A typical gradient elution (e.g., water/acetonitrile) for nonpolar analytes would be from initially very polar (high water content) to less polar (lower water content).

F. Detectors. Most are non-destructive; multiple detectors can be used to analyze the mobile phase nearly simultaneously.

1. Refractive index detectors (RID) are the only "universal" detectors. Changes in the index of refraction of the mobile phase (the signal) are sensed by passing a light beam thru the mobile phase twice at an angle away from the normal. Temperature control is very critical ($\pm 0.001^\circ\text{C}$ or better). This detector is not used with gradient elution because the mobile phase index of refraction changes. Sensitivity is poor and LODs are high. A non-destructive detector.
2. UV-VIS detectors are the most widely used detectors. The signal is absorbance. Most analytes absorb in the UV, some in the visible, so selectivity can be adjusted by choosing the right wavelength. The mobile phase must be chosen for transparency at the desired wavelengths. The mobile phase passes through a special cuvette with a volume of 1 - 10 μL . A single beam design is used with either a line source + filter or a continuum source + monochromator (being able to adjust the wavelength down to ca. 200 nm makes this detector nearly universal). LOD's depend on the molar absorptivity at the detector wavelength. The dynamic range is about 2 orders-of-magnitude. The UV-VIS detector is non-destructive and useful for gradient elutions.

REALLY expensive detectors use a polychromator with diode array and a computer to obtain a complete UV-VIS spectrum several times a second. This system greatly improves identification and even resolution. If the spectrum changes from the beginning to the end of the peak, then the peak contains more than one analyte.

Related detectors based on fluorescence or IR absorbance are available. The fluorescence detector is very sensitive and highly selective for fluorescent analytes.

3. Electrochemical detectors offer a combination of selectivity, low L.O.D. and very small detector volume. The mobile phase and analyte pass through a 3-electrode thin-layer electrochemical cell. The analyte is detected and quantified by hydrodynamic voltammetry. The signal is the current flowing through the WKG electrode. The detector is selective for oxidizable or reducible analytes. Selectivity is further adjusted by choosing the applied potential for the WKG electrode. Maximum sensitivity for an analyte is obtained for E_{appl} corresponding to the limiting current of the analyte voltammogram. Examples of organic analytes that can be oxidized are: phenols, catechols, aromatic amines, thiols, hydroquinones. Examples of analytes that can be reduced are: nitroaromatics, nitrosoamines, quinones, disulfides, pyridiniums.

The detector design is a thin-layer flow-thru cell. The WKG and CTR electrodes form walls of the cell, and the REF electrode is often located downstream. Typically the detector is operated in the amperometric mode - constant E_{appl} ; display current vs time. The current is proportional to analyte

concentration at all times. The current is also dependent on the flow rate. L.O.D.'s down to 10^{-9} M are possible with isocratic elution.

Review

Definitions: normal phase, reverse phase, isocratic and gradient elution, silanization.

Concepts: Sketch a liquid chromatograph, and choose a mobile phase, type of column, and detector; justify your choices of mobile phase and detector. How and why is dissolved air removed from the mobile phase? When are gradient elutions useful? What is a typical stationary phase for reverse phase HPLC? In what order will analytes elute in reverse phase HPLC? How do the RI, UV-VIS and electrochemical detectors work?

XXVIII. MASS SPECTROMETRY.

Reading:

SH&N, ch. 11, pp. 253-261; ch. 20, pp. 498-532.

A. Overview.

4. Advantages.

- (a) high specificity (ability to detect one substance or class of substances).
- (b) extremely sensitive with very low LOD's.
- (c) powerful structural analysis - can often identify a compound, or track isotopic labels.
- (d) micro-method (only tiny samples are required).
- (e) fast analysis time for a single species (< 1 s).
- (f) excellent 'detector' for chromatography (GC, LC) - identify eluted compounds as they emerge from the column.

The “hyphenated” methods (GC-MS, LC-MS) are very popular now in many areas: forensic analysis, biological and biochemical research, etc.

5. Basic module diagram:

Sample $\rightarrow$ *ion source* $\rightarrow$ *mass analyzer* $\rightarrow$ *detector* $\rightarrow$ readout

In the ion source, the sample is converted to gaseous ions at low pressure. The ions are separated according to their mass-to-charge ratio (**m/z**) in the mass analyzer (mass spectrometer). Ions striking the detector generate current pulses.

The middle 3 modules (in *italics*) require a high vacuum ($< 10^{-3}$ torr), so an expensive vacuum system is part of the hardware. Usually the vacuum pump is a turbomolecular pump - think of a jet engine whose blades are powered by a high speed electric motor running at 60,000 rpm.

6. The mass spectrum.

A mass spectrum (the output) is a plot of intensity (# ions/s) of a particular **m/z**. **m** is given in atomic mass units (amu, also known as daltons). For small molecules and most ion sources, **z** is +1. For proteins, **z** can be large (up to +50 or higher). **z** can also be negative depending on the

ion source and the nature of the molecule. For most work, the resolution of the **m/z** scale is 1 dalton. The highest peak is called the base peak; it is assigned an arbitrary intensity of 100. The vertical scale of relative abundance is simply a ratio of intensity of each peak to the base peak. The peak whose **m/z** corresponds to the molecular weight of the compound analyzed is the molecular ion peak. The molecular ion **M** is the ion formed by removing an electron (**z** = +1) from the molecule (recall that the mass of an electron is 2000 times smaller than the mass of a hydrogen atom). Peaks at lower **m/z** are due to fragments of the molecule (called daughter ions). The fragmentation pattern (i.e., the mass spectrum) is useful in identifying the molecule or the functional groups present in the molecule

B. Ion Sources.

(Instructor's note: all current ion sources are horribly inefficient. Only a tiny fraction of the sample is converted to gaseous ions entering the mass analyzer. There is room for invention here.)

There are two basic categories of ion sources: (a) gas-phase sources, which require that the sample is a vapor at temperatures less than 300°C (same as in gas chromatography); and (b) desorption sources, which are used for non-volatile or thermally sensitive compounds.

Ion sources are also categorized as hard or soft. A hard ion source causes extensive fragmentation of the molecule. There are many daughter ion peaks, and often the molecular ion peak is missing. A soft source causes little or no fragmentation. The molecular ion peak is often the base peak.

We will consider 4 ion sources: electron impact, chemical ionization, MALDI, and electrospray.

1. Electron Impact (EI)(sketch). The gaseous analyte is bombarded with electrons with a kinetic energy of 70 to 100 eV. The electrons knock more electrons out of molecules to produce cations:



Electrodes accelerate the cations towards the mass analyzer. Characteristics: a gas-phase source, a hard source, widely used. Libraries of mass spectra in books and computer data bases are usually based on this ion source. Useful with GC.

2. Chemical Ionization (CI). Same diagram as the EI source except a reagent gas (e.g., CH₄) is present at ca. 1 torr. The CH₄ is ionized by the electron beam and produces ions, some of which are strong proton donors or hydride extractors:



Again, electrodes accelerate the cations towards the mass analyzer. Characteristics: a gas-phase source, a soft source.

3. Matrix-Assisted Laser Desorption/Ionization (MALDI)(sketch). The analyte is mixed with a matrix compound, usually a solid aromatic compound with a carboxylic acid. The solid matrix is placed on a solid probe, inserted into the ionization chamber (under vacuum) and exposed to a short UV laser pulse. For reasons not yet understood, intact gaseous molecular ions of the analyte are formed. Characteristics: a desorption source, a very soft source (molecular ion only), very

useful for large biomolecules (proteins), can only be used with the TOF mass analyzer (see below), very expensive (requires a powerful laser).

7. Electrospray Ionization (ESI)(sketch). A sample (usually in aqueous solution) is fed to a metal tube held at several kilovolts with respect to a cylinder electrode. The high electric field causes small charged liquid droplets to form. Some of the droplets enter a heated capillary. The solvent evaporates, and excess charge ends up on the analyte. Pumped chambers with narrow apertures (skimmers) remove the lighter solvent molecules while the analyte ions pass through to the mass analyzer. Characteristics: a desorption source, a very soft source, molecular ions are often highly charged (high **z**), very useful for biomolecules, used as the interface between a LC and the mass analyzer (handout).

It is worth mentioning a special source for elemental MS analysis.

8. Inductively Coupled Plasma (ICP). Recall how the ICP torch works. Solids and liquids are converted to gaseous ions in the extremely hot temperatures of the plasma. The ions are admitted into the MS and analyzed. All structural information is lost. This is an excellent elemental analysis method - highly selective, very sensitive, good precision and accuracy.

C. Mass Analyzers.

The mass analyzer separates ions by their **m/z** ratio. Characterized by resolution ($R = m/\delta m$), transmission ($T = \% \text{ of ions of fixed } m/z \text{ reaching the detector}$) and speed (S) (time to acquire a mass spectrum).

Ex. $^{14}\text{N}_2$ has a mass of 28.006, while $^{12}\text{C}^{16}\text{O}$ has a mass of 27.995. What R is needed to distinguish these two molecules? $R = \frac{1}{2}(28.006 + 27.995)/(28.006 - 27.995) = 2540$

We will consider 3 mass analyzers: quadrupole, time-of-flight, and ion trap. Other important mass analyzers include the magnetic sector, the electrostatic sector, the double-focusing, and the ion cyclotron resonance designs.

1. Quadrupole mass analyzers (sketch). Ions of low kinetic energy (ca. 10 eV) enter via a circular aperture and travel down the long axis between 4 parallel poles. A combination of DC and AC voltages are applied to the poles with opposite poles having the same voltage. Only ions of a specific **m/z** follow a narrow helical path to the exit circular aperture; all other ions are thrown out and hit the poles. Mass scanning is performed by ramping both the DC and AC voltage amplitudes. Advantages of this design are (a) high S (**m/z** scans in a fraction of a second), (b) high T , and (c) relatively low cost. The disadvantages are (a) moderate resolution ($R < 1000$) and (b) limited mass range (up to **m/z** = 3000).
3. Time-of-flight (TOF) analyzers (sketch). The ion source must generate a short (nanosecond) pulse of ions with a fixed and well-defined kinetic energy. The ions drift along a long flight tube (ca. 1 meter in length). Lighter ions travel faster and arrive at the detector first. The advantages are (a) high S (a complete spectrum in 30 μs), (b) high T , and (c) no theoretical upper limit to **m/z** range (useful for high molecular weight analytes). The disadvantages are (a) low R , and (b) need for a special pulsed ion source (e.g., MALDI), a very fast detector and electronics.
4. Ion trap analyzer (sketch). Ions injected through one end cap are trapped by carefully programmed electric fields between two end cap electrodes and a ring electrode. Changing the electric fields

allows ions of increasing m/z to be ejected through the other end cap. The advantages are (a) high S, (b) high T, (b) low cost, and (d) smaller size than other designs. Their disadvantages are (a) a moderate R (< 1000) and (b) a limited mass range (up to $m/z = 2000$).

D. Detectors.

An ion striking a conducting surface with enough energy (ca. 10 keV) will kick out electrons. Heavier ions require greater energies to kick out electrons, so many detectors have acceleration grids just before the detector.

Electron multiplier detectors resemble PMTs and have high internal gain (up to 10^7) by using dynodes just like PMTs. The current output is related to the number of ions hitting the detector per second. These detectors are extremely fast and can be used with TOF mass analyzers.

Tandem MS (MS^n). The quadrupole and ion trap mass analyzers are capable of special methods that greatly increase selectivity (at great costs).

Triple quadrupole - three quadrupole units in series. Q_1 selects one molecular ion. Q_2 causes that molecular ion to collide with He atoms, resulting in fragmentation. Q_3 analyzes the daughter ions of the molecular ion.

An ion trap mass analyzer can do the same thing - select ions of one m/z (ejecting all other m/z ions); collide the selected ion with a neutral gas atom, and analyze the fragments.

Review

Definitions: mass-to-charge ratio, molecular ion, daughter ion, EI, CI, MALDI, ESI, TOF, resolution, speed, transmission.

Concepts: What are the advantages of mass spectrometry? What is plotted in a mass spectrum? What is the base peak? What are the differences between gas-phase and ion desorption sources, and between hard and soft ion sources? How are ions generated in EI, CI, MALDI and ESI ion sources (include sketches if appropriate)? What are their characteristics? Sketch each of the 3 types of mass analyzers (quadrupole, time-of-flight, and ion trap) and discuss how it works. What are their advantages and disadvantages? What is tandem MS?

Calculations: Resolution necessary for separating 2 close m/z peaks.

XXIX. CAPILLARY ZONE ELECTROPHORESIS.

(It is likely that this material will not be covered in lecture. I leave it here for your benefit. You will be informed if you will be tested on any of this material.)

Suggested reading:

SH&N, ch. 30, pp. 778-795.

Advantages: Rapid analyses (usually seconds to minutes); very high resolution (N over 10^5). Excellent method for separating small charged molecules in aqueous solutions. Often applied to bio-analytical problems.

Electrophoresis is the separation of charged species in an electric field. The analyte is dissolved in an electrolyte and a voltage is applied between 2 electrodes in the electrolyte. Ions migrate towards oppositely charged electrode. Ion migration velocity depends on the field gradient ($\delta V/\delta x$) and the viscosity of the electrolyte. Also, the charge of the ion (z) and its size also affect migration velocity, so ions starting from one point will eventually become separated.

Note: Gel electrophoresis is often used to separate proteins and other large biomolecules. The electrolyte is trapped in an inert polymer matrix (the gel). Larger molecules have greater difficulty squeezing through the polymer strands, and hence they migrate more slowly.

One problem limiting the resolution of bands is local heating caused by current flow. Heating effects are reduced if the dimensions of the electrophoresis experiment are reduced to a thin slab or, better still, a small capillary. A typical capillary used in CZE is made of pure silica (SiO_2) and has an internal diameter less than 100 μm .

If a silica capillary is filled with electrolyte, the 2 ends immersed in electrolyte with electrodes, and a high voltage applied to the electrodes, then the electrolyte will flow through the capillary. This phenomenon is known as electro-osmosis. The negative charge on the silica surface (controlled by electrolyte pH; higher pH $\rightarrow$ more negative surface charge) attracts the cations of the electrolyte towards the silica surface. The cations also migrate towards the negative electrode. Their migration pushes the electrolyte in the same direction. Because the pushing force is greatest at the walls of the capillary, the velocity of the electrolyte is nearly uniform across the diameter of the capillary (plug flow vs pressure flow - see Color Plate 27 in Harris). Plug flow reduces band broadening and contributes to the high N values of CZE.

Diagram of a CZE instrument. In the CZE experiment, a small (ca. 10 nL) amount of sample is injected into one end of the capillary using electro-osmotic injection or pressure injection. There are advantages and disadvantages to each mode of injection. Electro-osmotic injection gives a narrower band, but selects for analytes with higher mobility (which distorts the concentrations of the sample). Pressure injection gives a broader band, but gets every analyte onto the column at its proper concentration. After placing that capillary end into clean electrolyte, electro-osmosis is used to drive the sample towards the other end of the capillary (which is ca. 1 meter long). The analytes are separated in space by ion migration and reach the other end of the capillary (or a detector) at different times. The most common detection method is UV-VIS spectroscopy, but fluorescence, conductivity and electrochemical oxidation/reduction have been used to create detectors. At the more expensive end of the price scale, a CZE can be interfaced with a mass spectrometer to identify each component as it exits the capillary.

Neutral compounds can also be separated by adding surfactants to the electrolyte. The surfactants form micelles, globular clusters of molecules with a nonpolar interior and a charged surface. The neutral compounds partition into the micelles to varying degrees, and the micelles undergo ion migration. This method is known as micellar electrokinetic capillary chromatography (MECC).

Biomolecules (proteins, DNA fragments) can be separated if the capillary is filled with a gel electrolyte. Separations take hours instead of minutes, but excellent resolution is still achievable.