Analytical Chemistry Notes

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1 Introduction

Analytical chemistry is the science of obtaining, processing, and communicating information about the composition and structure of matter.

Analytical techniques include spectroscopy, chromatography, electrochemistry, etc.

1.1 Propagation of Error

Propagation of error is a method of estimating the uncertainty in a calculated quantity based on the uncertainties in the variables used to calculate it.

Suppose $x = f(a, b, c)$. We can write the total differential change as a sum of partial derivatives.

$$x = \frac{\partial f}{\partial a} da + \frac{\partial f}{\partial b} db + \frac{\partial f}{\partial c} dc \tag{1}$$

Now we want to express the above equation in terms of the standard deviation. The commonly accepted definition of standard deviation is

$$\sigma^2 = \frac{1}{N-1} \sum_{i=1}^{N} (x_i - \bar{x})^2 \tag{2}$$

If we assume that the errors are small, then we can rewrite Equation 1 as

$$(x_i - \bar{x}) = (a_i - \bar{a}) \frac{\partial x}{\partial a} + \left(b_i - \bar{b}\right) \frac{\partial x}{\partial b} + (c_i - \bar{c}) \frac{\partial x}{\partial c} \tag{3}$$

Thus, the standard deviation is

$$\sigma_x^2 = \frac{1}{N-1} \sum_{i=1}^{N} \left[(a_i - \bar{a}) \frac{\partial x}{\partial a} + \left(b_i - \bar{b}\right) \frac{\partial x}{\partial b} + (c_i - \bar{c}) \frac{\partial x}{\partial c} \right]^2 \tag{4}$$

Note that the cross terms in the summation will cancel each other out if the variables are linearly independent. Therefore,

$$\sigma_x^2 = \left(\frac{\partial x}{\partial a}\right)^2 \sigma_a^2 + \left(\frac{\partial x}{\partial b}\right)^2 \sigma_b^2 + \left(\frac{\partial x}{\partial c}\right)^2 \sigma_c^2. \tag{5}$$

2 UV-Visible Spectroscopy

Notes based off of Skoog's textbook.

UV-Visible Spectroscopy uses the amount of light a sample absorbs in the UV-Visible region of the electromagnetic spectrum to determine the concentration of an analyte. Concentration and absorption are related by the Beer-Lambert Law:

$$A = -\log(T) = \log\left(\frac{P_0}{P}\right) = \varepsilon b c \tag{6}$$

where A is the absorbance, T is the transmittance, P_0 is the incident power, P is the transmitted power, ε is the molar extinction coefficient, b is the path length, and c is the concentration.

2.1 Sources of Uncertainty

At concentrations of > 0.01 M, the solvent-solute interactions can affect the analyte concentration and absorptivity, and the refractivity index may change.

Deviations can also occur with a polychromatic light source when the extinction coefficient for two different wavelengths differ.

Similarly, stray radiation may lead to negative absorbance errors.

An intercept will occur in the calibration curve when the cells holding the analyte and blank solutions of not equal path length.

TABLE 13-3 Types and Sources of Uncertainties in Transmittance Measurements

Category	Characterized by[a]	Typical Sources	Likely To Be Important In
Case I	$s_T = k_1$	Limited readout resolution	Inexpensive photometers and spectrophotometers having small meters or digital displays
		Heat detector Johnson noise	IR and near-IR spectrophotometers and photometers
		Dark current and amplifier noise	Regions where source intensity and detector sensitivity are low
Case II	$s_T = k_2\sqrt{T^2 + T}$	Photon detector shot noise	High-quality UV-visible spectrophotometers
Case III	$s_T = k_3 T$	Cell positioning uncertainties	High-quality UV-visible and IR spectrophotometers
		Source flicker	Inexpensive photometers and spectrophotometers

[a] k_1, k_2, and k_3 are constants for a given system.

Figure 1: The types of uncertainties can be characterized by s_T, the standard deviation of transmittance.

2.2 Instrumentation

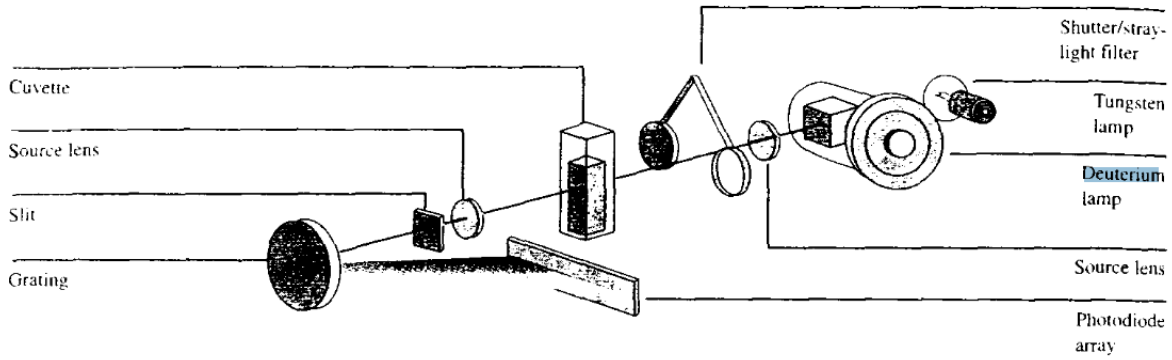

FIGURE 13-25 A multichannel diode-array spectrophotometer, the Agilent Technologies 8453. (Courtesy of Agilent Technologies, Palo Alto, CA.)

Figure 2: Light is emitted by a Tungsten and Deuterium lamp, and transmittance is measured by a spectrometer.

2.3 Why do different compounds absorb light at different wavelengths?

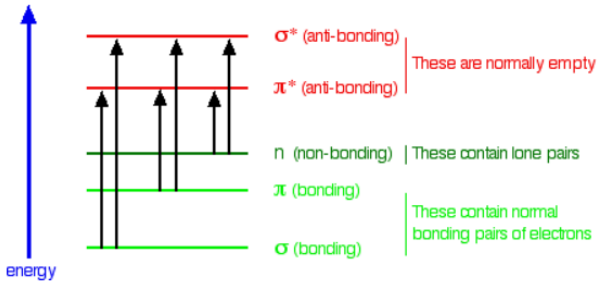

Figure 3: Different Energy Gaps.

Different compounds have different energy gaps. A photon with a wavelength of $\lambda = hc/E$ is required to jump the energy gap E. After it is absorbed, an electron will be excited, and energy can be released either radiatively (luminescence) or irradiatively (heat).

3 Fluorescence Techniques

Notes based off of Lakowicz's textbook.

Luminescence is the emission of light from any substance and can be divided into two categories: fluorescence and phosphorescence.

- **Fluorescence** occurs when the electron in the excited singlet state orbital returns to the ground state and emits a photon. The fluorescent lifetime is typically 10 ns.
- **Phosphorescence** is the emission of light from triplet excited state. The phosphorescent lifetimes are typically from milliseconds to seconds.

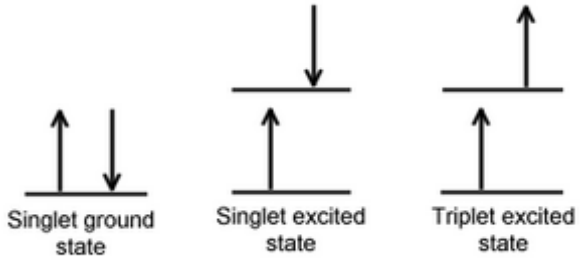

Figure 4: Excited singlet and triplet states. Not that the difference is that the electron spins are antiparallel in the singlet state and parallel in the triplet state.

3.1 Fluorescence Characteristics

- Fluorescence typically occurs at lower energies or longer wavelengths.
- Emission spectra are typically independent of the excitation wavelength.

Fluorescence lifetime and quantum yield are perhaps the most important characteristics of a fluorophore.

- **Quantum yield** is the ratio of the number of emitted photons to the number of absorbed photons.

$$Q = \frac{\Gamma}{\Gamma + k_{nr}} \tag{7}$$

where Γ is the emissive rate of the fluorophore and k_{nr} is the rate of nonradiative decay.

- **Fluorescence lifetime** is the average time a molecule spends in the excited state before returning to the ground state.

$$\tau = \frac{1}{\Gamma + k_{nr}} \tag{8}$$

Fluorescence quenching is any process which decreases the intensity of fluorescence. The process of emission requires more time than the process of absorption.

Fluorescence anisotropy is a phenomenon in which light emitted by a fluorophore has different intensity values along different axes of polarization

3.2 Fluorescence Resonance Energy Transfer (FRET)

Fluorescent Resonance Energy Transfer (FRET) is a process by which energy is transferred from a donor fluorophore to an acceptor fluorophore through long range dipole-dipole interactions.

The rate of energy transfer from a donor to an acceptor $k_T(r)$ is given by

$$k_T(r) = \frac{1}{\tau_D}\left(\frac{R_0}{r}\right)^6 \tag{9}$$

where τ_D is the decay time of the donor in the absence of acceptor, r is the distance between the donor and acceptor, and R_0 is the Förster distance. The Förster distance is the distance at which the energy transfer efficiency is 50%.

Förster distance can be calculated as

$$R_0^6 = \frac{9000 \ln(10)\kappa^2 Q_D}{128\pi^5 N n^4} J(\lambda). \tag{10}$$

Q_D is the quantum yield of the donor in the absence of acceptor. n is the refractive index of the medium, N is Avogadro's number, κ is the orientation factor, and $J(\lambda)$ is the overlap integral of the donor emission spectrum and the acceptor absorption spectrum.

$$J(\lambda) = \int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \tag{11}$$

$F_D(\lambda)$ is the corrected fluorescence intensity of the donor, and $\varepsilon_A(\lambda)$ is the extinction coefficient of the acceptor.

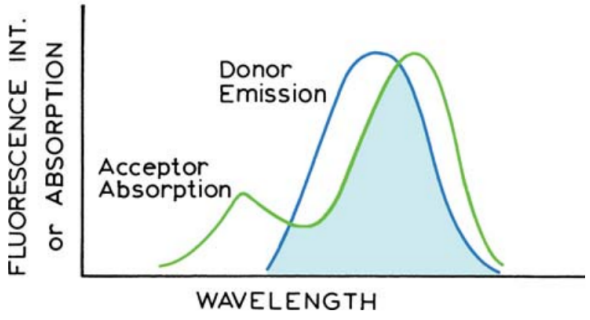

Figure 5: Spectral overlap of donor emission and acceptor absorption shaded in blue.

The efficiency of energy transfer (E) is the fraction of photons absorbed by the donor which are transferred to the acceptor.

$$E = \frac{k_T(r)}{\tau_D^{-1} + k_T(r)} = \frac{R_0^6}{R_0^6 + r^6} \tag{12}$$

The efficiency of energy transfer is typically measured using fluorescent intensities of the donor in the absence (F_D) and presence (F_{DA}) of acceptor.

$$E = 1 - \frac{F_{DA}}{F_D} = 1 - \frac{\int I_{DA}(t)dt}{\int I_D(t)dt} \tag{13}$$

3.3 Time-Correlated Single-Photon Counting (TCSPC)

There are two methods to measure fluorescence lifetimes: frequency domain and time domain. TCSPC is of the latter.

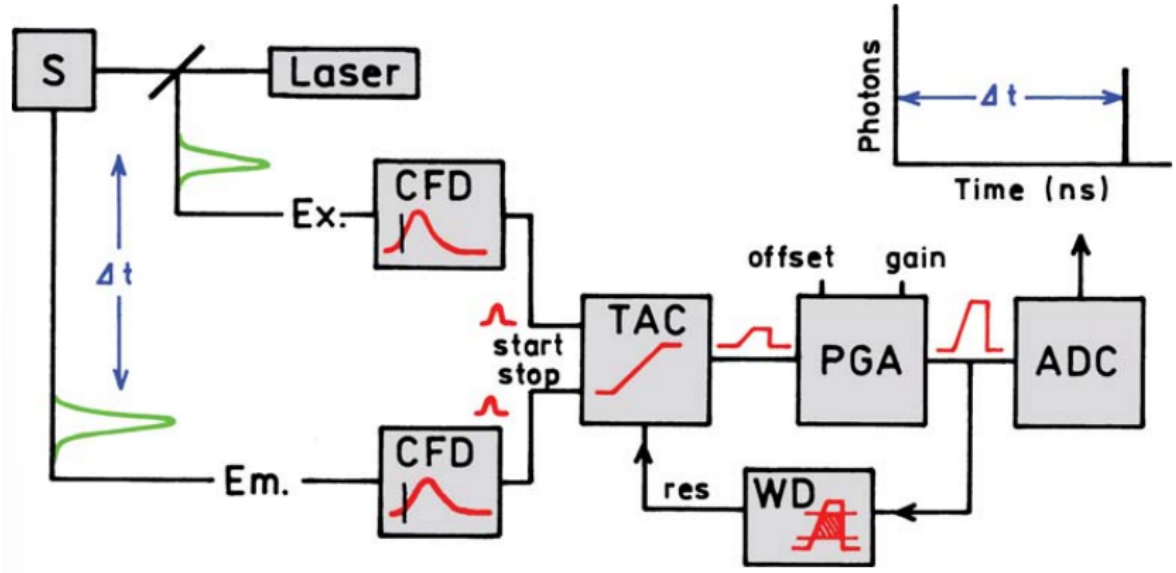

Figure 6: Electronic schematic for TCSPC.

In TCSPC, the sample is excited with a pulse of light, there are two constant function discriminators (CFD) which accurately measure the time of arrival of the photon for both excitation and emission. Only the first arriving photon is detected per excitation pulse because electronics are not fast enough to detect multiple photons within a short timespan. Then a time-to-amplitude converter (TAC) measures the time between the excitation and emission photons. A programmable gain amplifier (PGA) amplifies the signal, and the signal is converted to a numerical value by the analog to digital converter (ADC).

Count rates are limited to 1 photon per 100 excitation pulses to minimize the chance of two photons arriving per pulse (since we can only detect one at a time).

Normally before the sample is measured, the instrument response function (IRF) is measured with a zero-lifetime sample (usually Ludox) to determine the shortest time profile that can be measured by the instrument.

The final data is then fitted to a multiexponential decay curve which can be integrated after to calculate efficiency of energy transfer.

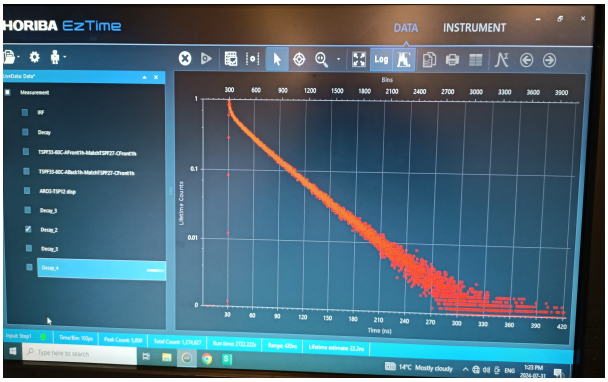

Figure 7: Example FRET data at Winnik Lab.

3.4 Fluorescence Spectroscopy

An **excitation spectrum** is the dependence of emission intensity, measured at a single emission wavelength, upon scanning the excitation wavelength (different from absorption spectra).

An **emission spectrum** is the wavelength distribution of an emission measured at a single constant excitation wavelength.

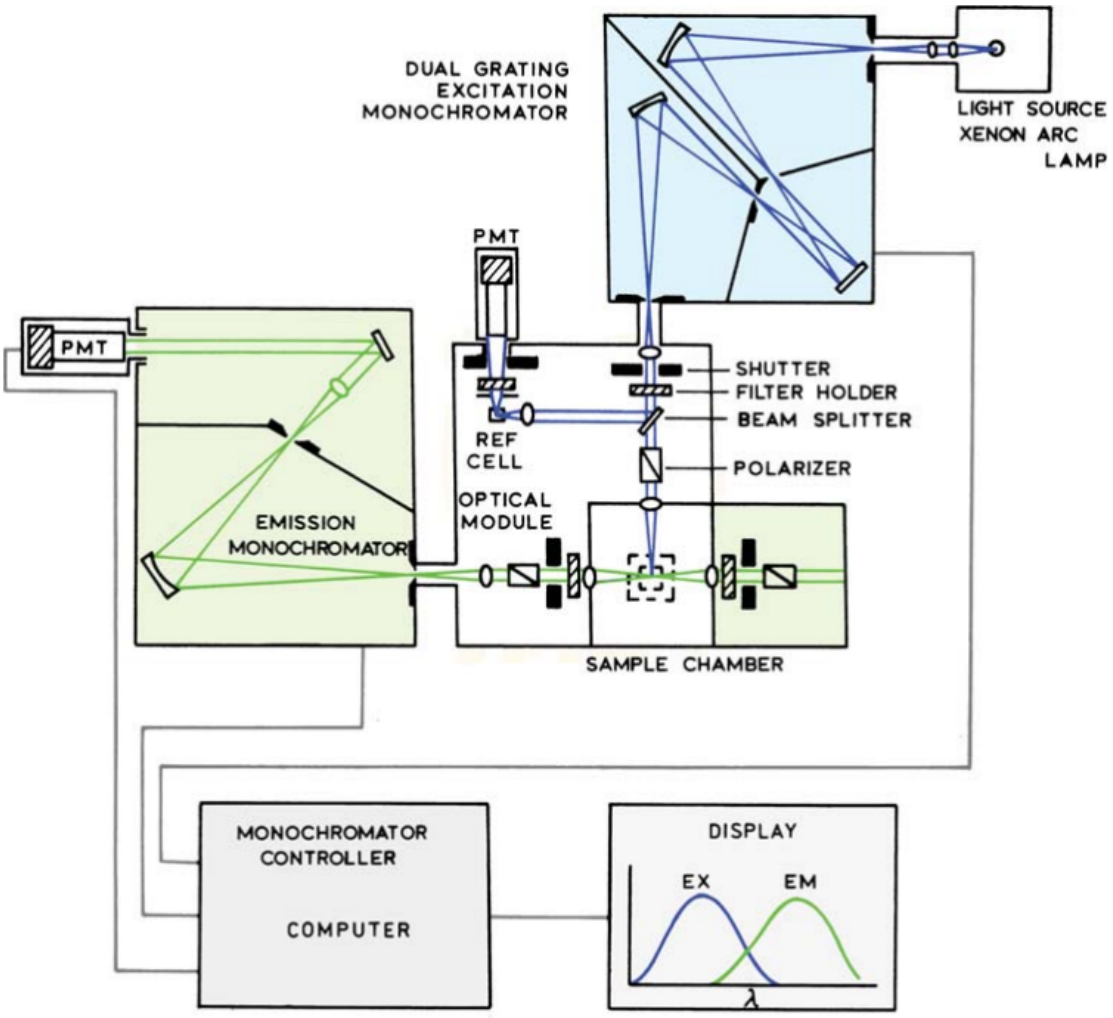

Figure 8: A schematic diagram of a spectrofluorometer.

Photomultiplier tubes (PMT) are used to detect the emitted light.

Monochromators are used to separate polychromatic light into its constituent wavelengths (usually diffraction gratings rather than prisms).