Lecture #4

Spectroscopy: Absorbance and Structure

(Skoog, Chapt. 14)
(pp. 329-345)

(Harris, Chapt. 19)
(pp.510-519, 523-530)

- Error

<table>
<thead>
<tr>
<th></th>
<th>Systematic Errors</th>
<th>Random Errors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>What?</strong></td>
<td>Fluctuations around true value</td>
<td></td>
</tr>
<tr>
<td><strong>Nature</strong></td>
<td>Predictable (consistently high of consistently low)</td>
<td>Unpredictable</td>
</tr>
<tr>
<td><strong>Causes</strong></td>
<td>Improper calibration of instrument (Instrumental, method, personal errors)</td>
<td>Difficulty taking measurements (hard to pin-point in most cases)</td>
</tr>
<tr>
<td><strong>Correction?</strong></td>
<td>Possible with calibrations</td>
<td>Can’t be corrected easily. However, statistics on errors may be helpful.</td>
</tr>
</tbody>
</table>
Recap L#3 (Aarthi's addendum)

- Uncertainty & precision
- Detection limits

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Smallest measurement that can be detected on an instrument (related to detection limit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selectivity</td>
<td>Ability of an instrument/method to only detect the target analyte in the presence of several other similar analytes.</td>
</tr>
<tr>
<td>Resolution</td>
<td>Smallest change in a measurable variable to which the instrument will respond (closeness to true value; better resolution if closer to true value)</td>
</tr>
</tbody>
</table>

\[
A = -\log_{10}(T) = -\log_{10}\left(\frac{I}{I_0}\right) \\
T = e^{-A} \\
A = acx = \epsilon cx \\
\]

-Beer-Lamberts Law

\[
C = \text{concentration (mg/L or M)} \\
X = \text{path length (cm)} \\
a \text{ and } \epsilon \text{ are both absorptivity coefficients when } C \text{ is expressed as mg/L or M respectively; } \epsilon \text{ most commonly referred to as molar absorptivity coefficient} \]
### Recap L#3 (Aarthi’s addendum)

<table>
<thead>
<tr>
<th>What?</th>
<th>Fluorescence</th>
<th>Phosphorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron spin</td>
<td>does not change in electron spin, which results in</td>
<td>there is a change in electron spin</td>
</tr>
<tr>
<td>Excited state duration</td>
<td>short-live electrons ($&lt;10^{-5}$ s) in the excited state of fluorescence</td>
<td>a longer lifetime of the excited state (second to minutes)</td>
</tr>
<tr>
<td>Wavelengths</td>
<td>Both occur at wavelengths longer than excited radiation</td>
<td></td>
</tr>
<tr>
<td>Examples</td>
<td>Fluorescent lights and neon signs, highlighter pens</td>
<td>Glow in the dark stars, paint used to make star murals.</td>
</tr>
</tbody>
</table>

Vieques, Puerto Rico (Bioluminescence Bay)  
(Bioluminiscence and phosphorescence are not the same!!!)
Let’s get clear on some interchangeably used terms here (Aarthi’s addendum)

**Spectroscopy** is the study of radiated energy and matter to determine their interaction, and it does not create results on its own.

**Spectrometry** is the application of spectroscopy so that there are quantifiable results that can then be assessed.

NIST definition of **Spectrophotometry**
"the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength. While relatively simple in concept, determining the reflectance or transmittance involves careful consideration of the geometrical and spectral conditions of the measurement."

**Spectrophotometry**
- “Procedure that uses light to measure chemical concentration” - Dr. Dave Reckhow

**Spectrometer**
- Produces, disperses and measures light

**Photometer**
- Detector that measures the amount of photons absorbed and send a signal to display.
Spectrophotometry

- “Procedure that uses light to measure chemical concentration” - Dr. Dave Reckhow

- Properties of Light
- Interaction of light with matter
- Atom & Light Energy

Properties of Light

- $\lambda$ (wavelength): crest to crest distance between waves
- $\nu$ (frequency, s$^{-1}$): number of complete oscillations that the wave makes each second
  
  1 oscillation/second = Hertz (Hz)

- $c$ (speed of light): 2.998 X 10$^8$ m/s

$E = h \nu$  

E = Energy carried by each photon  

h = Planck's constant (6.63$\times$10$^{-34}$ J.s)  

$\nu$ = Frequency (s$^{-1}$)
What happens when light strikes a sample?

- Some light is transmitted through the sample
- Some light is absorbed by the material
- Some light is reflected at each surface
- Some light is scattered to the side

**Beer-Lambert’s Law**

\[ A = \varepsilon \cdot l \cdot c \]

- \( A \): Absorbance of radiation
- \( \varepsilon \): Molar extinction coefficient or molar absorptivity (M\(^{-1}\).cm\(^{-1}\))
- \( l \): Path length (cm)
- \( c \): Concentration (M)

Interaction of radiation with matter
Review-Atom & Light Energy

Atom: Building block of a matter (protons (+), neutrons, electrons (-))
Protons+neutrons= nuclei
Neutral atom: protons=electrons

Ground State of an electron is the state of lowest energy for that electron.
Ionized electron formed as a result of loss or gain of electron

When an electron temporarily occupies an energy state greater than its ground state, it is in an excited state.

Electrons do not stay in excited states for very long - they soon return to their ground states, emitting a photon with the same energy as the one that was absorbed.
Electronic transitions

- Types of photon-absorbing electrons in organic molecule
  - Electrons that participate directly in bond formation between atoms
  - Non-bonding or unshared electrons that are localized about such atoms as oxygen, the halogens, sulfur, and nitrogen

- Types of transitions
  - $\sigma \rightarrow \sigma^*$
  - $\pi \rightarrow \pi^*$
  - $n \rightarrow \sigma^*$
  - $n \rightarrow \pi^*$

  Bonding ($\pi$, $\sigma$) (stabilize, low energy) & anti bonding orbitals ($\pi^*$, $\sigma^*$) (higher energy)

Review- Quantum numbers

- Principal quantum number ($n$)
  - Defines the size and the energy of an orbital ($n=1$, 2, 3, etc)
    - $n=1$ (ground state)
    - $n>1$ (excited state)

- Angular quantum number ($l$)
  - Defines the shape of the orbital ($l=0$ to $n-1$)
    - $l=0$ (s), $l=1$ (p), $l=2$ (d), $l=3$ (f), $l=4$ (g)

- Magnetic quantum number ($m$)
  - Defines the orientation of the orbital ($m=-l$ to $+l$)

- Spin magnetic quantum number ($m_s$)
  - Defines the direction of an electron ($m_s=-1/2$ or $+1/2$)
    - $+1/2$ for spin up
    - $-1/2$ for spin down
Review-Electron configuration

- Orbital with the lowest energy is filled first (1s orbital), orbital in the second shell (n=2) is filled next and so on…
  - $^6$C 1s$^2$ 2s$^2$ 2p$^2$
  - 1st shell has 1 orbital (1s)
  - 2nd shell has 4 orbital (1s and 3p)

- Molecular Orbital (interaction between atomic orbitals creates a bonding and antibonding molecular orbitals)
  - H$_2$
  - O$_2$

Energy Absorption & Bonding

- A=absorbance
- F=fluorescence
- P=phosphorescence
- IC=internal conversion
- ISC=intersystem crossing
- R=vibrational relaxation
The most applications of absorption spectroscopy to organic compounds are based upon transitions for n or \( \pi \) electrons to the \( \pi^* \) excited state. Both transitions require the presence of unsaturated functional groups to provide the \( \pi \) orbitals.

**Difference between two types of transitions:** \( \pi \rightarrow \pi^* \) & \( n \rightarrow \pi^* \)

- Molar absorptivity for peak associated with \( n \rightarrow \pi^* \) transition are low (10 to 100 \( M^{-1}.cm^{-1} \)). For \( \pi \rightarrow \pi^* \) transition, \( \varepsilon \) range from 1000 to 10000 \( M^{-1}.cm^{-1} \).

- Effect of solvent
  - Peaks associated with \( n \rightarrow \pi^* \) transition are shifted to shorter wavelength (hypsochromic shift) with increasing polarity of solvent
  - Peaks associated with \( \pi \rightarrow \pi^* \) transition are shifted to longer wavelength (bathochromic shift) with increasing polarity of solvent
Absorbance Spectra

- Nitrobenzene in aqueous solution

\[ \text{Absorbance Spectrum for a 0.1 mM solution} \]

- Heavily conjugated with 4 resonance forms

\[ \text{Molar Absorptivity on a log scale} \]

Graph from: Schwarzenbach et al., 1993

Terminology

- **Absorbance** \((A)\) a measure of the amount of radiation that is absorbed
- **Band** Term to describe a uv-vis absorption which are typically broad.
- **Chromophore** Structural unit responsible for the absorption
- **Molar absorptivity** \((\epsilon)\), absorbance of a sample of molar concentration in 1 cm cell.
- **Extinction coefficient** An alternative term for the molar absorptivity
- **Path length** \((l)\) the length of the sample cell in cm
- **Beer-Lambert Law** \(A = \epsilon.l.c\) \((c = \text{concentration in moles / litre})\)
- **\(\lambda_{\text{max}}\)** The wavelength at maximum absorbance
- **\(\epsilon_{\text{max}}\)** The molar absorbance at \(\lambda_{\text{max}}\)
- **HOMO** Highest Occupied Molecular Orbital
- **LUMO** Lowest Unoccupied Molecular Orbital
**Bathochromic Shift**

- Ethylene
  \[ \lambda_{\text{max}} = 190 \text{ nm} \]
- Butadiene
  \[ \lambda_{\text{max}} = 220 \text{ nm} \]
- Benzene
  \[ \lambda_{\text{max}} = 255 \text{ nm} \]
- Nitrobenzene
  \[ \lambda_{\text{max}} = 270 \text{ nm} \]

**Conjugation**

- Impact of double bonds in conjugation with aromatic ring
- More $\pi \rightarrow \pi^*$ transitions
- Example
  - Benzene
  - Styrene

From: Schwarzenbach et al., 1993
Heteroatoms

- Impact of heteroatoms
  - $n \rightarrow \pi^*$ transitions
    - Longer $\lambda_{\text{max}}$
  - Example
    - Trans-stilbene
    - Azobenzene

Conjugation revisited

- Impact of increasing conjugation
  - $\pi \rightarrow \pi^*$ transitions
    - $\lambda_{\text{max}}$ increases $\sim 30$ nm per conjugated bond
      - Bathochromic shift
  - Examples
    - Naphthalene
    - Anthracene
    - Phenanthrene

From: Schwarzenbach et al., 1993
- More Examples
  - Naphthacene
  - Benz(a)anthracene

From: Schwarzenbach et al., 1993
Geometry

- 1,2-Naphthoquinone
- 1,4-Naphthoquinone

From: Schwarzenbach et al., 1993

pH speciation: Acids

- Deprotonation leads to delocalization of negative charge
  - Bathochromic shift
- Examples
  - 4-Nitrophenol
  - 4-Nitrophenolate

From: Schwarzenbach et al., 1993
pH Speciation: Bases

- Protonation causes loss of available “n” electrons
  - Hypsochromic shift
- Examples
  - Aniline
  - Anilinium ion

From: Schwarzenbach et al., 1993

Background NOM

- Specific Absorbance of water samples from several Swiss lakes and rivers

From: Schwarzenbach et al., 1993
S::CAN

- Field deployable diode array spectrophotometer in a probe-like configuration
- Can be submersed in flowing water or fitted with a flow-through cell
- Produces a full UV-Vis spectrum
- Algorithms tailored to estimate other parameters
- Good surrogate for DOC
  - especially when the character of the DOC is reasonably constant
- A very good surrogate for THMFP, HAAFP
  - takes into account reactivity of DOC as well as amount of DOC
- Oxidation processes (ozonation) disrupt relationships between UV and DOC or THMFP

Commercial field probe

Derivative Spectroscopy

- Derivatives can be used in various algorithms

Thomas & Burgess, 2007
Derivative Spectra

- Derivative of absorbance with respect to wavelength
- Some features
  - the 2nd derivative shows a negative peak at the $\lambda_{\text{max}}$
  - the 4th derivative shows a positive peak at the $\lambda_{\text{max}}$

Applications of Derivative Spectroscopy 1

- Resolution of overlapping spectral bands
- Spectra must be relatively free of noise
Applications of Derivative Spectroscopy 2

- Removal of background interference, e.g., scattering
  - +9.2% error in peak height
  - -1.1% error in max to min of 1st derivative

- To next lecture