Lecture #5

Molecular Spectroscopy: Instrument Design & Errors
(Skoog, Chapt. 13 & 6; pp.312-325, 131-134, 143-189)

Atomic Spectroscopy: Basics
(Skoog, Chapt. 8 & 9; pp.192-203, 206-227)

(Harris, Chapt. 21) (pp.584-592)
(Harris, Chapt. 22) (pp.615-635)
Spectrophotometers

- 4 major components

Light Source

Wavelength Selector

Sample Holder

Detector
Light Sources

- Line sources
  - Discrete wavelengths
  - Used for AA and will be discussed later

- Continuous Sources
  - Used for molecular absorption and fluorescence
  - Visible: tungsten lamp
  - UV: deuterium lamp
Tungsten Lamp

- Black body radiation
- High temp
  - (2870 °C)

\[ \text{output} \sim (\text{voltage})^4 \]
\[ \sim (\text{temperature})^4 \]
- Therefore good voltage control is quite important
Deuterium Arc Lamp

- Deuterium provides more power than hydrogen

- Where

\[ H_2 + E_e \rightarrow H_2^* \rightarrow H' + H'' + h\nu \]

- About 1000 hr half-life

\[ h\nu = E_e - E_{H'} - E_{H''} \]
Reflection Gratings

- Each groove behaves as a point source
Reflection Gratings 1

- Each groove behaves as a point source
Reflection Gratings 2

• Full destructive interference occurs when:
  • The difference in length of adjacent light paths is exactly equal to the wavelength of that light or some multiple of the wavelength.
  
\[ n\lambda = a - b \]

• And since
  
\[ a = d\sin\theta \]
\[ b = d\sin\phi \]

• Then
  
\[ n\lambda = d(\sin\theta - \sin\phi) \]
Grating Monochromator
# Sample Cells

<table>
<thead>
<tr>
<th>Window</th>
<th>Wavelength Range (nm)</th>
<th>Letter Code</th>
<th>Lot #s</th>
<th>Color Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical Glass</td>
<td>360 - 1000</td>
<td>OG</td>
<td></td>
<td>yellow</td>
</tr>
<tr>
<td>Near-UV Glass or Special Optical Glass</td>
<td>300 - 1000</td>
<td>OS or SG</td>
<td>180's</td>
<td>green</td>
</tr>
<tr>
<td>Standard Silica</td>
<td>220 - 2500</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supracil Quartz or Quartz UV</td>
<td>165 - 2600, 2850 - 3600</td>
<td>QS or UV</td>
<td>280's</td>
<td>blue</td>
</tr>
<tr>
<td>Infracil Quartz or Quartz IR</td>
<td>220 - 3600</td>
<td>QI or IR</td>
<td>300's</td>
<td>red</td>
</tr>
</tbody>
</table>

![Graph showing transmittance (%)](image_url)
• Photomultipliers
  • consists of a photocathode and a series of dynodes in an evacuated glass enclosure.
    • When a photon of sufficient energy strikes the photocathode, it ejects a photoelectron due to the photoelectric effect. The photocathode material is usually a mixture of alkali metals, which make the PMT sensitive to photons throughout the visible region of the electromagnetic spectrum. The photocathode is at a high negative voltage, typically -500 to -1500 volts. The photoelectron is accelerated towards a series of additional electrodes called dynodes. These electrodes are each maintained at successively less negative potentials. Additional electrons are generated at each dynode.
    • This cascading effect creates $10^5$ to $10^7$ electrons for each photoelectron that is ejected from the photocathode. The amplification depends on the number of dynodes and the accelerating voltage. This amplified electrical signal is collected at an anode at ground potential, which can be measured.
Detectors 2

- Phototubes
  - similar to PMTs, but consist of only a photocathode and anode.
    - Since phototubes do not have a dynode chain to provide internal amplification, they are used in less sensitive applications such as absorption spectrometers.
Simple Spectrophotometer

- Spectronic 20
• Turner 350
Double Beam Spectrophotometer

Diagram showing the components of a double beam spectrophotometer, including a monochromator, rotatable grating, collimating mirror, entrance and exit slits, entrance and exit toroid mirrors, and the double-beam photometer with reference and sample cuvettes, chopper, and detector.
Errors

- Bandwidth and linearity
Errors

- Bandwidth and linearity
Errors

- Band width and resolution

![Graph showing wavelength and percent transmittance with different bandwidths.](image-url)
Errors

- Effect of stray light on absorbance spectra
- Example of stray light in a spectrophotometer with glass optics (A) compared to quartz optics (B)
Impacts of Stray light

- Causes increase in signal to detector that is not really “transmitted light”

Thomas & Burgess, 2007
Errors, cont.

- Effect of stray light on linearity

![Graph showing the effect of stray light on absorbance with concentration]
The full picture

- Exact area of greatest accuracy depends on instrument
Errors

- Precision vs. Abs

![Graph showing precision vs. absorbance](image)
Optrodes

- **Optical Fiber construction**

- **Generic Instrument Design**
  - Probe type photometer
  - Called an optrode when combined with chemical detectors that produce an absorbing product with analyte
    - Fig 7.9, pg 198
Optrodes (cont.)

- Fiber sensor used for measuring penicillin in blood
  - Harris pg. 597
Figure 16-1  Infrared absorption spectrum of a thin polystyrene film recorded with a modern infrared spectrophotometer. Note that the abscissa scale changes at 2000 cm$^{-1}$. 
Fluorescence & Luminescence

Three types of Luminescence methods are:

- (i) molecular fluorescence
- (ii) phosphorescence
- (iii) chemiluminescence

In each, molecules of the analyte are excited to give a species whose emission spectrum provides information for qualitative or quantitative analysis.
Description

- **Fluorescence**: absorption of photon, short-lived excited state (singlet), emission of photon.
- **Phosphorescence**: absorption of photon, long-lived excited state (triplet), emission of photon.
- **Chemiluminescence**: no excitation source – chemical reaction provides energy to excite molecule, emission of photon.
- Luminescence: High sensitivity → strong signal against a dark background.
- Used as detectors for HPLC.
Quantum Yield

Quantum Yield, $\phi = \frac{\text{total # luminescing molecules}}{\text{total # of excited molecules}}$

$\phi = \frac{k_f}{k_f + k_i + k_{ec} + k_{ic} + k_{pd} + k_d}[k = \text{rate constant}]$
Structure effects

- Low–energy $\pi \rightarrow \pi^*$ (aromatic): most intense fluorescence.
- Heterocycles do not fluoresce; heterocycles fused to other rings fluoresce. Heteroatom increases ISC then $\phi_f$ decreases.
- Conjugated double bond structures exhibit fluorescence.
- Structural rigidity (e.g., naphthalene or fluorene vs biphenyl). Flexibility increases then $\phi_f$ decreases.
- Temperature: increase fluorescence intensity with decreasing T (reduce number of deactivating collisions).
fluorene

biphenyl
• Solvent: increase fluorescence with increased viscosity (decreased likelihood of external conversion – radiationless deactivation)

• Heavy atoms such as I, Br, Th increases ISC as a consequence $\Phi_f$ decreases

• pH: Increased resonance structures (protonation or deprotonation) $\rightarrow$ stable excited state and greater quantum yield

• pH can also influence emission wavelength (changes in acid dissociation constant with excitation)
resonance forms of aniline

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<table>
<thead>
<tr>
<th>Ion</th>
<th>Reagent</th>
<th>Wavelength, nm</th>
<th>LOD, μg/mL</th>
<th>Interferences</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Absorption</td>
<td>Fluorescence</td>
<td></td>
</tr>
<tr>
<td>Al(^{3+})</td>
<td>Alizarin garnet R</td>
<td>470</td>
<td>500</td>
<td>0.007</td>
</tr>
<tr>
<td>F(^{-})</td>
<td>Quenching of Al(^{3+}) complex of alizarin garnet R</td>
<td>470</td>
<td>500</td>
<td>0.001</td>
</tr>
<tr>
<td>B(_4)O(_7)(^{2-})</td>
<td>Benzoin</td>
<td>370</td>
<td>450</td>
<td>0.04</td>
</tr>
<tr>
<td>Cd(^{2+})</td>
<td>2-(o-Hydroxyphenyl)-benzoxazole</td>
<td>365</td>
<td>Blue</td>
<td>2</td>
</tr>
<tr>
<td>Li(^{+})</td>
<td>8-Hydroxyquinoline</td>
<td>370</td>
<td>580</td>
<td>0.2</td>
</tr>
<tr>
<td>Sn(^{4+})</td>
<td>Flavanol</td>
<td>400</td>
<td>470</td>
<td>0.1</td>
</tr>
<tr>
<td>Zn(^{2+})</td>
<td>Benzoin</td>
<td>—</td>
<td>Green</td>
<td>10</td>
</tr>
</tbody>
</table>

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Protein fluorescence

**Diagram:**
- Two graphs showing spectral properties of different amino acids:
  - Left graph: Absorbance (ε) vs. Wavelength (nm) with peaks for TRP, TYR, and PHE.
  - Right graph: Fluorescence Intensity vs. Wavelength (nm) with peaks for TRP and TYR.
Protein-labeling Probes

Absorption and fluorescence emission spectra of dansyl cadaverine in methanol.

τ = 10 -15 ns
Φ = 0.1 - 0.3

Absorption and fluorescence emission spectra of dansyl cadaverine in methanol.
Protein-labeling Probes

Absorption and fluorescence emission spectra of goat anti-mouse IgG labeled with fluorescein-5-isothio-cyanate in pH 8.0 buffer.

\[ \tau = 4.5 \text{ ns} \]
\[ \Phi = 0.3 - 0.85 \]
**Coumarin Fluorescence Probes**

- **C153**
  - Localizes in PPO hydrophobic/dry core
  - $clogP = 4.08$

- **C102**
  - Localizes in PPO/PEO regions (water?)
  - $clogP = 3.67$

- **C343$^-$/Na$^+$**
  - Located primarily in wet phases
  - $clogP = -1.09$
• Aq. PEO$_{109}$-PPO$_{41}$-PEO$_{109}$
• 5 w/v % solution forms micelles
• Probes localize in different regions
  – Experience different electrical environments
**Fluorescence - EEMs**

**Excitation-Emission Matrices:** Fluorescence intensity across the range of emission wavelengths while also scanning across excitation wavelengths.

Contour plots of 7 components identified from the complete F-EEMs dataset.

Baghoth et al., 2011

- Terrestrial Humic
- Marine & Terrestrial Humic
- Terrestrial/Anthropogenic Humic
- Amino Acid
- Marine & Terrestrial Humic
- Correlates well with some NOM properties, but fundamental understanding is still not good
Location of EEM peaks (symbols) based on literature reports and operationally defined excitation and emission wavelength boundaries (dashed lines) for five EEM regions

Chen et al., 2003
Algal Organics

*Microsystis aeruginosa*

I: protein-like
II: protein-like
III: humic-like
IV: protein-like
V: humic-like

Fang et al., 2010
Figure 16-2 Types of molecular vibrations. Note: + indicates motion from the page toward the reader; – indicates motion away from the reader.
Atomic Spectrophotometry

- Use
  - Analysis of metals
  - Very sensitive

- Three types
  - Absorption (AAS)
    - Flame and electrothermal (furnace)
  - Emission (AES)
    - Often used with plasma
  - Fluorescence
Atomic Absorption Spectrophotometers

- Sample holder is replaced with an atomizer
Atomic Absorption

- General

- Flame
• To next lecture