Chapter 14 – Applications of Molecular Absorption Spectrometry

Read: pp 367–380

Problems: 14–1, 2, 8

\[ A(\lambda) = \varepsilon(\lambda)bC \]

\( \varepsilon \) values in UV/Vis molecular absorption spectrometry range from 0 to \( 10^5 \)!

\( \varepsilon = 8.7 \times 10^{19} \text{ PA} \)

Capture cross-section area \( \sim 10^{-15} \text{ cm}^2 \)

Probability of energy-absorbing transition 0–1
Absorbance Measurements in Gas vs. Liquid Phase

Solvent matters!!!

Polar solvents tend to obliterate the fine structure.

As a rule, same solvent system should be used when comparing absorption spectra for identification purposes.

Absorption spectra for vapor shows much fine structure (e.g., numerous rotational and vibrational states associated with excited electronic state are visible.

In condensed state, less rotational freedom so rotational states not observed.

When chromophore is surrounded by solvent molecules, energies of vibrational levels are modified in a nonuniform way. Energy of a given state appears as a broad peak.
Effect of $\lambda_{\text{eff}}$

Peak heights and peak separation are distorted at wider bandwidths.

Loss of resolution accompanies wider slit widths.

Spectra for qualitative applications should be measured with minimum slit width.

**FIGURE 14-7** Spectra for reduced cytochrome c at four spectral bandwidths. (1) 20 nm, (2) 10 nm, (3) 5 nm, and (4) 1 nm. (Courtesy of Varian, Inc., Palo Alto, CA.)
Absorbing Species

$M + h\nu \rightarrow M^*$  
Excitation event $(10^{-9} \text{ s})$

$M^* \rightarrow M + \text{heat or light}$  
Relaxation event

UV/Vis – excitation of bonding electrons!

Can be used for quantitative purposes and for functional group (type of bonding) information.
Absorbing Organic Molecules Containing $\sigma$, $\pi$ and n Electrons

Absorbing functional groups = chromophores

Olefins and aromatics

$\sigma \rightarrow \sigma^* < 185$ nm

$n \rightarrow \sigma^* 150-250$ nm

$\pi \rightarrow \pi^* 200-700$ nm

Conjugation = delocalization lowers energy level of $\pi^*$ orbital

<table>
<thead>
<tr>
<th>Chromophore</th>
<th>Example</th>
<th>Solvent</th>
<th>$\lambda_{max}$(nm)</th>
<th>$\epsilon_{max}$</th>
<th>Type of Transition</th>
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<tbody>
<tr>
<td>Alkene</td>
<td>C\textsubscript{4}H\textsubscript{13}CH=CH\textsubscript{2}</td>
<td>n-Heptane</td>
<td>177</td>
<td>13,000</td>
<td>$\pi \rightarrow \pi^*$</td>
</tr>
<tr>
<td>Alkyne</td>
<td>C\textsubscript{2}H\textsubscript{11}C=CH\textsubscript{3}</td>
<td>n-Heptane</td>
<td>178</td>
<td>10,000</td>
<td>$\pi \rightarrow \pi^*$</td>
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<td></td>
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<td>196</td>
<td>2,000</td>
<td>$\pi \rightarrow \pi^*$</td>
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<td>225</td>
<td>160</td>
<td>$\pi \rightarrow \pi^*$</td>
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<tr>
<td>Carbonyl</td>
<td>CH\textsubscript{3}C=CH\textsubscript{3}</td>
<td>n-Hexane</td>
<td>186</td>
<td>1,000</td>
<td>$n \rightarrow \sigma^*$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>280</td>
<td>16</td>
<td>$n \rightarrow \pi^*$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>293</td>
<td>12</td>
<td>$n \rightarrow \pi^*$</td>
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<tr>
<td>Carboxyl</td>
<td>CH\textsubscript{3}COH</td>
<td>Ethanol</td>
<td>204</td>
<td>41</td>
<td>$n \rightarrow \pi^*$</td>
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<tr>
<td>Amido</td>
<td>CH\textsubscript{3}CNH\textsubscript{2}</td>
<td>Water</td>
<td>214</td>
<td>60</td>
<td>$n \rightarrow \pi^*$</td>
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<tr>
<td>Azo</td>
<td>CH\textsubscript{3}N=NC\textsubscript{3}H\textsubscript{3}</td>
<td>Ethanol</td>
<td>339</td>
<td>5</td>
<td>$n \rightarrow \pi^*$</td>
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<tr>
<td>Nitro</td>
<td>CH\textsubscript{3}NO\textsubscript{2}</td>
<td>Isooctane</td>
<td>280</td>
<td>22</td>
<td>$n \rightarrow \pi^*$</td>
</tr>
<tr>
<td>Nitroso</td>
<td>C\textsubscript{4}H\textsubscript{9}NO</td>
<td>Ethyl ether</td>
<td>300</td>
<td>100</td>
<td>$n \rightarrow \pi^*$</td>
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<tr>
<td>Nitrate</td>
<td>C\textsubscript{2}H\textsubscript{5}ONO\textsubscript{2}</td>
<td>Dioxane</td>
<td>270</td>
<td>12</td>
<td>$n \rightarrow \pi^*$</td>
</tr>
</tbody>
</table>
Typical Absorption Spectra for Organic Molecules

\[ n \rightarrow \pi^* \] are often shifted to shorter wavelengths (hypsochromic or blue shift) with increasing solvent polarity.

\[ \pi \rightarrow \pi^* \] are often shifted to longer wavelengths (bathochromic or red shift) with increasing solvent polarity.

**Figure 14-4** Ultraviolet spectra for typical organic compounds.
Absorption Involving d and f Electrons

Crystal-Field Theory

First and second transition-metal series. Compounds are colored as absorption occurs at visible wavelengths (300-700 nm).
Chapter 15 - Molecular Luminescence Spectrometry

Read: pp 399-417

Problems: 15-1, 3, 7

Light emission process!

Radiative or non-radiative decay/relaxation.

Fluorescence and phosphorescence vs. chemiluminescence

*Figure 15-1* Partial energy diagram for a photoluminescent system.
Process of Excitation and Emission

• **Absorption of light** - $10^{-15}$ s and related to $\varepsilon$

• **Vibrational relaxation** – excess vibrational energy in solution immediately lost in solution due to collisional deactivation, $10^{-12}$ s.

• **Internal conversion** – intermolecular process by which a molecule passes to a lower energy electronic state *without* emission of light. Overlap of vibrational energy levels in two electronic energy levels.

• **External conversion** – deactivation of an excited electronic state by interaction and energy transfer between the excited molecule and solvent or other solutes.

• **Intersystem crossing** – process in which spin of an excited electron is reversed and change in multiplicity results. Most common when vibrational manifold overlap exists and when the molecule has a heavy atom substituent (e.g., Br, I).

• **Fluorescence and Phosphorescence** – relaxation of an excited state via light emission. Time scales range from $10^{-6}$ s to 100’s s.
Quantitative Aspects of Fluorescence Measurements

\[ F = 2.3\Phi \varepsilon bC P_0 \]

- \( \Phi \) = quantum efficiency = \# molecules emitting/total \# molecules excited
- \( \varepsilon \) (L/mol-cm) and \( b \) (cm) have their usual meanings
- \( P_0 \) in incident radiant power density (watts/cm\(^2\))

- Linear relationship, \( F = KC \)

- *Self-absorption* and *self-quenching* cause negative deviations from linearity (i.e., reduced fluorescence intensity).

- \( \Phi \) increases with lower temperature, increased structural rigidity, \( \pi \rightarrow \pi^* \) transition, and can be affected by solvent type and pH.

- Electron donating groups (NH\(_2\), OH) tend to enhance fluorescence while electron withdrawing groups (Cl, COOH) tend to inhibit it.
Excitation and Emission Spectra

Resonance vs. non-Resonance Fluorescence

Non-Radiant losses result in red shift in fluorescence.

Excitation at fixed wavelength and recording the emission spectra.

Figure 15-2 Fluorescence excitation and emission spectra for a solution of quinine.
Basic Design of a Simple Fluorometer

Figure 15-4 Components of a fluorometer or a spectrofluorometer.
Figure 15-6 A typical fluorometer. (Courtesy of Farrand Optical Co., Inc.)

Figure 15-7 A spectrofluorometer. (Courtesy of SLM Instruments, Inc., Urbana, IL.)
Chapter 7 – Components of Optical Instruments

Read pp. 164-173; 180-190; 191-200
Problems: 1, 2, 3, 6, 16, 19
Configuration of an instrument for an absorption measurement.

Source of EMR (perturbation Signal) → Sample Holder → Wavelength Selector → Detector → Signal Readout

- Broad band (λ’s) or line source (λ)
- Transparent and chemically inert
- Separate or isolate the specific wavelength
- Transducer that provides an electrical signal proportional to light intensity
- Computer or other readout device

Remember: All light intensity loss must be due to absorbance by the analyte. Therefore, two measurements are always necessary: one with the analyte present and a background (without the analyte).
**Figure 20-3** Intensity of a tungsten filament at 3200 K and a deuterium arc lamp.
Detectors or Transducers

- Devices that record intensity changes in the incident light and convert these intensity changes to a proportional electrical signal.

- \( I_{ph} \sim \) light intensity \( S = kP + k_d \)

- Single channel or multichannel types.

- Sensitivity, stability, dark current, can it respond to more than one wavelength simultaneously, etc.

- Phototubes, photodiodes vs. photomultiplier tubes vs. charge transfer devices (CCD’s).
**Types of Detectors**

Single Channel

*Figure 7-27* A phototube and accessory circuit. The photocurrent induced by the radiation causes a potential drop across $R$, which is then amplified to drive a meter or recorder.

\[ I_{ph} \text{ (photocurrent)} = kP \text{ (radiant power)} \]
Types of Detectors

Single Channel

\[ I_{ph} \text{ (photocurrent)} = kP \text{ (radiant power - amplified)} \]
Multichannel Detector (Multiple Wavelengths Simultaneously)
Figure 20-13  (a) Schematic cross-sectional view of photodiode array. (b) Photograph of array with 1024 elements, each 25 μm wide and 2.5 mm high. The central black rectangle is the photosensitive area. The entire chip is 5 cm in length. [Courtesy Oriel Corporation, Stratford, CT.]
Figure 20-15  Schematic representation of a charge coupled device. (a) Cross-sectional view, indicating charge generation and storage in each pixel. (b) Top view, showing two-dimensional nature of an array. An actual array is about the size of a postage stamp.