Chapter 13
An Introduction to Ultraviolet/Visible Molecular Absorption Spectrometry
Absorption measurements based upon ultraviolet and visible radiation find widespread application for the quantitative determination of a large variety of species.

Ordinarily, the concentration of an absorbing analyte is linearly related to absorbance as given by Beer's law:

\[ A = -\log T = \log \frac{P_0}{P} = \varepsilon bc \]

- **Beer’s Law:**

  \[ A = \text{absorbance} \]
  \[ \varepsilon = \text{molar absorptivity [M}^{-1} \text{cm}^{-1}] \]
  \[ c = \text{concentration [M]} \]
  \[ P_0 = \text{incident power} \]
  \[ P = \text{transmitted power (after passing through sample)} \]
Transmittance and absorbance, as defined in Table 13-1, cannot normally be measured in the laboratory because the analyte solution must be held in a transparent container or cell.
Reflection occurs at the two air-wall interfaces as well as at the two wall-solution interfaces.

The resulting beam attenuation is substantial, as we demonstrated in Example 6-2, where it was shown that about 8.5% of a beam of yellow light is lost by reflection in passing through a glass cell containing water.

In addition, attenuation of a beam may occur as a result of scattering by large molecules and sometimes from absorption by the container walls.

FIGURE 13-1 Reflection and scattering losses with a solution contained in a typical glass cell. Losses by reflection can occur at all the boundaries that separate the different materials. In this example, the light passes through the air-glass, glass-solution, solution-glass, and glass-air interfaces.
Measurement of Transmittance and Absorbance:

The power of the beam transmitted by the analyte solution is usually compared with the power of the beam transmitted by an identical cell containing only solvent. An experimental transmittance and absorbance are then obtained with the equations.

$P_0$ and $P$ refers to the power of radiation after it has passed through the solvent and the analyte.

$$T = \frac{P_{\text{solution}}}{P_{\text{solvent}}} = \frac{P}{P_0}$$

$$A = \log \frac{P_{\text{solvent}}}{P_{\text{solution}}} \approx \log \frac{P_0}{P}$$
• **Derivation of Beer’s Law:**

Sample cell with absorbing molecules
\[ \text{d} P \propto P \quad \text{Incremental power lost } \propto \text{ power in}; \text{ i.e., increase power in, increase power absorbed} \]
\[ \text{d} P \propto \text{d} b \quad \text{Longer pathlength, greater number of molecules in incremental slice and more power absorbed} \]
Therefore, \[ \text{d} P \propto P \text{d} b \quad \text{d} P = -kP \text{d} b \]
\[ k = \text{proportionality constant (function of } \lambda, c) \]
\[ \text{negative sign: because power is lost (i.e., absorbed)} \]
Rearrange:
\[ \frac{dP}{P} = -kdb \]
Integrate: 
\[ \int_{P_0}^{P} \frac{1}{P} dP = -k \int_{0}^{b} db \]

\[ \ln P - \ln P_0 = -kb - (-k)(0) \]

\[ \ln \frac{P}{P_0} = -kb \]

Factor out concentration part of k: \( k = k''c \)

\[ \ln \frac{P}{P_0} = -k''bc \]

Convert fraction (remove –sign) and change In to log:

\[ (1/2.303)k'' = \varepsilon \]

\[ \log \frac{P_0}{P} = \frac{1}{2.303}k'':bc \]

\[ A = \log \frac{P_0}{P} = \varepsilon bc \]
Beer’s law applies to a medium containing more than one kind of absorbing substance. Provided there is no interaction among the various species, the total absorbance for a multicomponent system is given by

\[ A_{\text{total}} = A_1 + A_2 + \ldots + A_n = \varepsilon_1 bc_1 + \varepsilon_2 bc_2 + \ldots + \varepsilon_n bc_n \]

where, the subscripts refer to absorbing components 1, 2, …, n.
Assumptions in derivation of Beer’s Law

- Incident radiation is monochromatic (all molecules absorb light of one $\lambda$).
- Absorbing molecules act independently of one another i.e., low $c$.
- Pathlength is uniform (all rays travel the same distance in sample).
- No scattering.
- Absorbing medium is optically homogeneous.
- Incident beam is not large enough to cause saturation.
- All rays should be parallel to each other and perpendicular to the surface of the medium.
13B-2 Limitations (deviations) to Beer’s Law

* Real Limitations
  High concentration > 0.01 M
  • the extent of solute-solvent interactions, solute-solute interactions, or hydrogen bonding can affect the analyte environment and its absorptivity.

* Chemical Deviations
  • Analyte dissociates, associates or reacts to give molecule with different absorption characteristics (e.g., pH-dependent indicators)
    – Example 13-1

* Instrumental Deviations
  – Polychromatic radiation
  – Stray Radiation
**Instrumental Deviations:**

- **In the presence of Polychromatic radiation (i.e., light of more than one \( \lambda \))**

\[
A_{\text{meas}} = \log \left( \frac{P'_0 + P''_0}{P' + P''} \right)
\]

Where \( P' \) and \( P'' \) are powers for \( \lambda' \) and \( \lambda'' \), respectively
- Negative deviation = lower absorbance than predicted because higher transmittance
- Higher T because molecules don’t absorb one \( \lambda \) as well as other

The absorber has the indicated molar absorptivities at the two wavelengths \( \lambda' \) and \( \lambda'' \).
The effect of polychromatic radiation on Beer's law. In the spectrum on the left, the absorptivity of the analyte is nearly constant over band A from the source. Note in the Beer's law plot at the bottom that using band A gives a linear relationship. In the spectrum, band B corresponds to a region where the absorptivity shows substantial changes. In the lower plot, note the dramatic deviation from Beer's law that results.

To avoid deviations, it is advisable to select a wavelength band near the wavelength of maximum absorption where the analyte absorptivity changes little with wavelength.
Instrumental Deviations:

-In the presence of Stray radiation

- The radiation exiting from a monochromator is usually contaminated with small amounts of scattered or stray radiation. This radiation, commonly called *stray light*, is defined as radiation from the instrument that is outside the nominal wavelength band chosen for the determination.

- This stray radiation often is the result of scattering and reflection off the surfaces of gratings, lenses or mirrors, filters, and windows.

- The wavelength of stray radiation often differs greatly from that of the principal radiation and, in addition, the radiation may not have passed through the sample.
**Instrumental Deviations:**

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**In the presence of Stray radiation**

$$A' = \log \left( \frac{p'_0 + P_s}{P' + P_s} \right)$$

$P_s =$ power from stray radiation

Extra light hits detector $\rightarrow$

higher $T$; lower $A$

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FIGURE 13-6 Apparent deviation from Beer's law brought about by various amounts of stray radiation. Note that the absorbance begins to level off with concentration at high stray-light levels. Stray light always limits the maximum absorbance that can be obtained because when the absorbance is high, the radiant power transmitted through the sample can become comparable to or lower than the stray-light level.
13.C Effect of Slit Width on Absorbance Measurements

- Figure 13-8 illustrates the loss of detail that occurs when slit widths are increased from small values on the left to larger values in the middle and right. In this example, the absorption spectrum of benzene vapor was obtained at slit settings that provided effective bandwidths of 1.6, 4, and 10 nm.

- For qualitative studies, the loss of resolution that accompanies the use of wider slits is often important because the details of spectra are useful for identifying species. **Narrow slits widths are required to resolve complex spectra**
quantitative measurement of narrow absorption bands requires using narrow slit widths or, alternatively, very reproducible slit-width settings. Unfortunately, a decrease in slit width by a factor of 10 reduces the radiant power by a factor of 100 because the radiant power is proportional to the square of the slit width.

- There is thus a trade-off between resolution and signal-to-noise ratio.
- For quantitative measurements, slit width needs to be kept large enough to provide high signal throughput.
Instrumentation

• Light source
• $\lambda$ - selection
• Sample container
• Detector
• Signal processing
• Light Sources (commercial instruments)
  – D$_2$ lamp (UV: 160 – 375 nm)
  – W lamp (vis: 350 – 2500 nm)
Deuterium and Hydrogen Lamps

Tungsten Filament Lamps

(a)

(b)

(c)
• \( \lambda \) - selection (monochromators)
• Sample holders
  – Cuvettes (\( b = 1 \) cm typically)
  – Glass (Vis)
  – Fused silica (UV 350 nm)
• Detectors
  – Photodiodes
  – PMTs
Types of Instruments

• **Single beam**
  – Place cuvette with blank (i.e., solvent) in instrument and take a reading \( \rightarrow 100\% \ T \)
  – Replace cuvette with sample and take reading \( \rightarrow \% \ T \) for analyte (from which absorbance is calc’d)

• **Double beam (most commercial instruments)**
  – Light is split and directed towards both reference cell (blank) and sample cell
  – Two detectors; electronics measure ratio (i.e., measure/calculate absorbance)
  – Advantages:
    • Compensates for fluctuations in source and drift in detector
    • Better design for continuous recording of spectra
Single beam

double beam in space

double beam in time

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Multichannel Instruments

- Photodiode array detectors used (multichannel detector, can measure all wavelengths dispersed by grating simultaneously).
- Advantage: scan spectrum very quickly “snapshot” < 1 sec.
- Powerful tool for studies of transient intermediates in moderately fast reactions.
- Useful for kinetic studies.
- Useful for qualitative and quantitative determination of the components exiting from a liquid chromatographic column.
Multichannel Instruments
Instruments for the Visible Region.

(a) Digital readout

(b) Diagram of optical components:
- Solid-state detector
- Tungsten lamp
- Sample
- Field lens
- Entrance slit
- Occluder
- Exit slit
- Objective lens
- Grating
- Wavelength cam
- Light control
FIGURE 13·21 Schematic of a typical manual double-beam spectrophotometer for the UV-visible region.
FIGURE 13.22 Schematic of the Varian Cary 100 double-beam spectrophotometer for the UV-visible region
FIGURE 13-25 A multichannel diode-array spectrophotometer, the Agilent Technologies 8453.
Chapter 14: Applications of UV-Vis Molecular Absorption Spectrometry

Characteristics of UV/Vis Methods:

- Wide applicability to organic and inorganic systems
- Sensitivities to $10^{-4}$ to $10^{-7}$ M
- Moderate to high selectivity
- Good accuracy, about 1-3% relative uncertainty
- Ease and convenient data acquisition
UV/Vis Absorbance:

- Results from excitation of bonding electrons. So, can correlate wavelength of absorption peaks with types of bonds.
- Types of electronic transitions relative to UV/Vis absorbance:
  1) p, s, and n electrons
  2) d and f electrons
  3) Charge-transfer electrons

Absorbing Electrons:

Electrons that contribute to absorbance in organic molecules:

1) Those that directly participate in bond formation between atoms and are associated with more than one atom.

2) Nonbonding or unshared outer electrons largely localized about atoms like oxygen, sulfur, nitrogen, halogens.
Most applications of absorption spectroscopy to organic molecules are based on \( n \) or \( p \) to \( p^* \) transition which require unsaturated functional groups.

**TABLE 14-2 Absorption Characteristics of Some Common Chromophores**

<table>
<thead>
<tr>
<th>Chromophore</th>
<th>Example</th>
<th>Solvent</th>
<th>( \lambda_{\text{max}} ) (nm)</th>
<th>( \epsilon_{\text{max}} )</th>
<th>Type of Transition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkene</td>
<td>( C_6H_{13}CH=CH_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_5H_{11}C=CC=CH_3 )</td>
<td>( n )-Heptane</td>
<td>178</td>
<td>10,000</td>
<td>( \pi \rightarrow \pi^* )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>196</td>
<td>2,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>225</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>Carbonyl</td>
<td>( \overset{\ddagger}{CH_3}C=CH_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>( \overset{\ddagger}{CH_3}CH )</td>
<td>( n )-Hexane</td>
<td>180</td>
<td>Large</td>
<td>( n \rightarrow \sigma^* )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>293</td>
<td>12</td>
<td>( n \rightarrow \pi^* )</td>
</tr>
<tr>
<td>Carboxyl</td>
<td>( CH_3COH )</td>
<td>Ethanol</td>
<td>204</td>
<td>41</td>
<td>( n \rightarrow \pi^* )</td>
</tr>
<tr>
<td>Amido</td>
<td>( CH_3CNH_2 )</td>
<td>Water</td>
<td>214</td>
<td>60</td>
<td>( n \rightarrow \pi^* )</td>
</tr>
<tr>
<td>Azo</td>
<td>( CH_3N=NC\text{H}_3 )</td>
<td>Ethanol</td>
<td>339</td>
<td>5</td>
<td>( n \rightarrow \pi^* )</td>
</tr>
<tr>
<td>Nitro</td>
<td>( CH_3NO_2 )</td>
<td>Isooctane</td>
<td>280</td>
<td>22</td>
<td>( n \rightarrow \pi^* )</td>
</tr>
<tr>
<td>Nitroso</td>
<td>( C_4H_9NO )</td>
<td>Ethyl ether</td>
<td>300</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Nitrate</td>
<td>( C_2H_5ONO_2 )</td>
<td>Dioxane</td>
<td>665</td>
<td>20</td>
<td>( n \rightarrow \pi^* )</td>
</tr>
</tbody>
</table>

Effect of solvents in reducing fine structure in absorbance spectra

Figure 6-19  Some typical ultraviolet absorption spectra.

- If chromophores are separated by more than one single bond, absorbance of multichromophores in a single organic molecule are approximately additive.

### Table 14-3 Effect of Multichromophores on Absorption

<table>
<thead>
<tr>
<th>Compound</th>
<th>Type</th>
<th>$\lambda_{\text{max}}$(nm)</th>
<th>$\epsilon_{\text{max}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH$_3$CH$_2$CH$_2$CH$\equiv$CH$_2$</td>
<td>Olefin</td>
<td>184</td>
<td>~10,000</td>
</tr>
<tr>
<td>CH$_2$$\equiv$CHCH$_2$CH$_2$CH$\equiv$CH$_2$</td>
<td>Diolefin (unconjugated)</td>
<td>185</td>
<td>~20,000</td>
</tr>
<tr>
<td>H$_2$C$\equiv$CHCH$\equiv$CH$_2$</td>
<td>Diolefin (conjugated)</td>
<td>217</td>
<td>21,000</td>
</tr>
<tr>
<td>H$_2$C$\equiv$CHCH$\equiv$CHCH$\equiv$CH$_2$</td>
<td>Triolefin (conjugated)</td>
<td>250</td>
<td>—</td>
</tr>
<tr>
<td>CH$_3$CH$_2$CH$_2$CH$_2$CCH$_3$</td>
<td>Ketone</td>
<td>282</td>
<td>27</td>
</tr>
<tr>
<td>CH$_2$$\equiv$CHCH$_2$CH$_2$CCH$_3$</td>
<td>Unsaturated ketone (unconjugated)</td>
<td>278</td>
<td>30</td>
</tr>
<tr>
<td>CH$_2$$\equiv$CHCCH$_3$</td>
<td>$\alpha,\beta$-Unsaturated ketone (conjugated)</td>
<td>324</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>219</td>
<td>3,600</td>
</tr>
</tbody>
</table>

Absorption of $d$ and $f$ electrons:

- Most transition-metal ions absorb in UV/Vis.
- Lanthanides and actinides have narrow, well-defined, characteristic absorption peaks.
- Ions or complexes from first and second transition series tend to absorb in all oxidation states, tend to be broad bands that are dependent on chemical environment.

Sample spectra from lanthanide ions

Effect of Solvent on Absorption Spectra

- Polar solvents eliminate fine detail. Nonpolar solvent keep spectral details more similar to gas phase measurements.

Absorbance Spectra of Mixtures:

- The absorbance of a solution at a given wavelength is the sum of the absorbances of the individual components.

\[
A_1 = \varepsilon_{M_1} b c_M + \varepsilon_{N_1} b c_N \\
A_2 = \varepsilon_{M_2} b c_M + \varepsilon_{N_2} b c_N
\]

➢ To analyze the mixture, molar absorptivities for M and N are first determined at wavelengths \( \lambda_1 \), and \( \lambda_2 \) with sufficient concentrations of the two standard solutions to be sure that Beer's law is obeyed over an absorbance range that encompasses the absorbance of the sample.

➢ Note that the wavelengths selected are ones at which the molar absorptivities of the two components differ significantly. Thus, at \( \lambda_1 \) the molar absorptivity of component M is much larger than that for component N.

➢ The greatest accuracy is obtained by choosing wavelengths at which the differences in molar absorptivities are large.

Photometric Titration:

- Plot absorbance (corrected for change in volume) as function of volume of titrant. Analyte, titrant, product, or indicator must absorb. High accuracy since multiple measurements are pooled.
- Photometric titrations are ordinarily performed with a spectrophotometer or a photometer that has been modified so that the titration vessel is held stationary in the light path.