SPECTROSCOPY

Light interacting with matter as an analytical tool
Electronic Excitation by UV/Vis Spectroscopy:

- **X-ray:** core electron excitation
- **UV:** valance electronic excitation
- **IR:** molecular vibrations
- **Radio waves:** Nuclear spin states (in a magnetic field)
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Different Spectroscopies

- UV-vis – electronic states of valence e/d-orbital transitions for solvated transition metals
- Fluorescence – emission of UV/vis by certain molecules
- FT-IR – vibrational transitions of molecules
- FT-NMR – nuclear spin transitions
- X-Ray Spectroscopy – electronic transitions of core electrons
Spectroscopies in the UV-Vis region

- UV-vis (molecular) absorption spectroscopy
- Solvated metal complexes
- Molecular fluorescence
- Atomic absorption
- Atomic Emission
UV-vis (molecular) absorption spectroscopy

- Quantitative/Beer’s Law
- Valence electronic transitions
- Molecular orbital theory
- Effect of conjugation
- Broadness of the spectra (solvent effect and superimposition of vibrational/rotational levels)
- Instrumentation
- Molecular Probes/biokits based on visible absorption spectroscopy
- Examples of some molecular probes
Quantitative Spectroscopy

• Beer’s Law

\[ A_{\lambda_1} = e_{\lambda_1}bc \]

- \( e \) is molar absorptivity (unique for a given compound at \( \lambda_1 \))
- \( b \) is path length
- \( c \) concentration
Beer’s Law

- \( A = -\log T = \log\left(\frac{P_0}{P}\right) = ebc \)
- \( T = \frac{P_{\text{solution}}}{P_{\text{solvent}}} = \frac{P}{P_0} \)
• $A = -\log T = \log \left( \frac{P_0}{P} \right) = ebc$
  – $e$ is molar absorptivity (L/mol•cm)
  – $B$ is the path length (cm)
  – $T$ is the transmittance
  – $T = \frac{P_{\text{solution}}}{P_{\text{solvent}}} = \frac{P}{P_0}$
• Works for monochromatic light
• Compound $x$ has a unique $e$ at different wavelengths
Characteristics of Beer’s Law Plots

- One wavelength
- Good plots have a range of absorbances from 0.010 to 1.000
- Absorbances over 1.000 are not that valid and should be avoided
- 2 orders of magnitude
Standard Practice

- Prepare standards of known concentration
- Measure absorbance at $\lambda_{\text{max}}$
- Plot $A$ vs. concentration
- Obtain slope
- Use slope (and intercept) to determine the concentration of the analyte in the unknown
Typical Beer’s Law Plot

\[ y = 0.02x \]
UV-Vis Spectroscopy

• UV- organic molecules
  – Outer electron bonding transitions
  – conjugation
• Visible – metal/ligands in solution
  – d-orbital transitions
  – Dyes – very high level of conjugation
• Instrumentation
Characteristics of UV-Vis spectra of Organic Molecules

• Absorb mostly in UV unless highly conjugated
• Spectra are broad, usually too broad for qualitative identification purposes
• Excellent for quantitative Beer’s Law-type analyses
• The most common detector for an HPLC
Molecules have quantized energy levels: ex. electronic energy levels.

\[ \Delta E = h\nu \]

Q: Where do these quantized energy levels come from?
A: The electronic configurations associated with bonding.

Each electronic energy level (configuration) has associated with it the many vibrational energy levels we examined with IR.
Broad spectra

• Overlapping vibrational and rotational peaks
• Solvent effects
Molecular Orbital Theory

• Fig 18-10
Molecular Orbital for $\text{O}_2$

\[ \sigma^* \]

\[ \pi^* \]

\[ \sigma \]

\[ \pi \]

\[ \sigma^* \]

\[ \sigma \]

\[ 2s \]

\[ 2p \]
Ethane

\[ \sigma \rightarrow \sigma^* \]

\[ \lambda_{\text{max}} = 135 \text{ nm} \quad (\text{a high energy transition}) \]

Absorptions having \( \lambda_{\text{max}} < 200 \text{ nm} \) are difficult to observe because everything (including quartz glass and air) absorbs in this spectral region.
Example: ethylene absorbs at longer wavelengths:
\[ \lambda_{\text{max}} = 165 \text{ nm} \ \varepsilon = 10,000 \]
The $n$ to $\pi^*$ transition is at even lower wavelengths but is not as strong as $\pi$ to $\pi^*$ transitions. It is said to be “forbidden.” Example:

Acetone:  
$n-\sigma^*$  \(\lambda_{\text{max}} = 188 \text{ nm} \);  \(\varepsilon = 1860\)  
\(n-\pi^*\)  \(\lambda_{\text{max}} = 279 \text{ nm} \);  \(\varepsilon = 15\)
\[
\begin{align*}
\text{C-C} & \quad \sigma \rightarrow \sigma^* \quad 135 \text{ nm} \\
\text{C=O} & \quad \pi \rightarrow \pi^* \quad 165 \text{ nm} \\
\text{C-O-H} & \quad n \rightarrow \sigma^* \quad 183 \text{ nm} \quad \text{weak} \\
\text{C=O} & \quad \pi \rightarrow \pi^* \quad 150 \text{ nm} \\
& \quad n \rightarrow \sigma^* \quad 188 \text{ nm} \\
& \quad n \rightarrow \pi^* \quad 279 \text{ nm} \quad \text{weak}
\end{align*}
\]
Conjugated systems:

Preferred transition is between Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO).

Note: Additional conjugation (double bonds) lowers the HOMO-LUMO energy gap:

Example:

1,3 butadiene: \( \lambda_{\text{max}} = 217 \text{ nm} \); \( \varepsilon = 21,000 \)

1,3,5-hexatriene \( \lambda_{\text{max}} = 258 \text{ nm} \); \( \varepsilon = 35,000 \)
Similar structures have similar UV spectra:

\[ \lambda_{\text{max}} = 238, 305 \text{ nm} \quad \lambda_{\text{max}} = 240, 311 \text{ nm} \quad \lambda_{\text{max}} = 173, 192 \text{ nm} \]
Lycopene:

\[
\lambda_{\text{max}} = 114 + 5(8) + 11*(48.0-1.7*11) = 476 \text{ nm}
\]

\[
\lambda_{\text{max}}(\text{Actual}) = 474.
\]

A solution of lycopene appears orange because it absorbs blue
Color wheel

- red (640 - 700 nm)
- orange (600 - 640 nm)
- yellow (560 - 600 nm)
- violet (400 - 450 nm)
- blue (450 - 480 nm)
- green (450 - 560 nm)
Yellow food coloring
Tartrazine

\[ \lambda_{\text{max}} = 427 \text{ nm} \]
Blue food coloring
Brilliant Blue

\[ \lambda_{\text{max}} = 628 \text{ nm} \]
Solvated Metal Ions

• The Spatial arrangements of ligands around a solvated metal ion causes distortion of the d-orbital
• Consequence: d-orbital splitting
• The \( \Delta E \) is often in the visible range of the spectrum
Octahedral Geometry
d-orbital splitting

Max. absorbs around 490 nm, solution appears pale pink

\[ \Delta E = \frac{hc}{\lambda} \]

Degenerate D-orbitals of naked Co

d-orbitals of hydrated Co\(^{2+}\)

Octahedral Configuration
Molecular Probes or TAGs

- Chemical reagents that have a detectable handle that can selectively bind to analyte
- Example/ BCA Total protein assay
  - combines reduction of Cu$^{2+}$ to Cu$^+$ by protein in an alkaline medium with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu$^+$) by bicinchoninic acid
  - Biuret rxn; chelation complex between amino groups and Cu$^+$ (light blue)
  - Cu$^+/BCA$ forms a purple complex, intense absorption at 562 nm
  - Microplate reader
Instrumentation

• Fixed wavelength instruments
• Scanning instruments
• Diode Array Instruments
Fixed Wavelength Instrument

- LED serve as source
- Pseudo-monochromatic light source
- No monochrometer necessary/ wavelength selection occurs by turning on the appropriate LED
- 4 LEDs to choose from

![Diagram showing a sample, beam of light, LEDs, and photodyode]
sources

• Tungten lamp (350-2500 nm)
• Deuterium (200-400 nm)
• Xenon Arc lamps (200-1000 nm)
Monochromator

- Bragg's law, $n\lambda = d(\sin i + \sin r)$
- Angular dispersion, $\frac{dr}{d\lambda} = \frac{n}{d(\cos r)}$
- Resolution, $R = \frac{\lambda}{\Delta \lambda} = nN$, resolution is extended by concave mirrors to refocus the divergent beam at the exit slit
Sample holder

• Visible; can be plastic or glass
• UV; you must use quartz
Single beam vs. double beam

• Source flicker
Diode array Instrument
Advantages/disadvantages

• Scanning instrument
  – High spectral resolution (63000), $\lambda/\Delta\lambda$
  – Long data acquisition time (several minutes)
  – Low throughput

• Diode array
  – Fast acquisition time (a couple of seconds), compatible with on-line separations
  – High throughput (no slits)
  – Low resolution (2 nm)
High Performance Liquid Chromatography (HPLC)

- Separation of mixtures
- Because UV-VIS spectra are so broad, components of a mixture need to be separated prior to detection
- Marriage between HPLC and UV/vis detector
  - HPLC separates components
  - Each component can be detected and quantified by UV-VIS detector