UV/Visible Spectroscopy

Introduction: Importance

Theory

Beer’s Law

Chromophores

Environmental Effects

  Monomers

  Biopolymers

Example of Application: RNA Stability
Electronic Transitions

Excited States (Photochemistry):
Photosynthesis
Photoresponse
Enzyme Mechanisms
Uses:
Analytical
Monitor Global Conformation
(Stability, Kinetics)

Large Energies:
500 nm → 57 kcal/mol
260 nm → 110 kcal/mol
Particle in a Box

Quantum Mechanical

\[ E_n = \frac{h^2 n^2}{8ml^2} \]

m = mass of electron

l = size of box

\[ \Delta E = h\nu = hc/\lambda \]

As size of box ↑, \( \Delta E \) ↓, so \( \lambda \) of absorption ↑
Intensity $\rightarrow$ Transition Probability

\[
\frac{dP_e}{dt} = B_{g\rightarrow e} I(\nu)
\]

\[
B_{g\rightarrow e} \propto \left| \langle \Psi_e | \vec{\mu} | \Psi_g \rangle \right|^2
\]

Electric Dipole Transition Moment

“This integral describes the ability of light to distort a molecule in state \([g]\) so as to produce elements that resemble state \([e]\).”  C & S, Vol. II, p. 168.

\[
\mu_{g\rightarrow e}^2 \propto \int \frac{\varepsilon(\lambda)d\lambda}{\lambda}
\]

\(\mu\) has fixed orientation relative to structure of molecule.

Classical Analogy:

\[
\vec{\mu}_{ind} = \vec{\alpha} \cdot \vec{E}
\]
Bonds, Orbitals, & Selection Rules

\( \pi \ \sigma^* \) bonds too high in E
\( \pi \ \pi^* \) bonds accessible w/ UV
\( \pi \) At least one component of Transition Dipole must be non-zero
\( \pi \ \pi \rightarrow \pi^* \) Strong
\( \pi \ n \rightarrow \pi^* \) Weak (Transition Dipole ~ 0, transition forbidden)
• Spin: \( \Delta S = 0 \)
Beer’s Law

\[ A = \log \frac{I_0}{I} = \varepsilon Cl \]

\( \frac{I}{I_0} = 0.99 \rightarrow 0.01, \ A = 0.005 \rightarrow 2.0 \)
\( \frac{I}{I_0} = 0.95 \rightarrow 0.05, \ A = 0.02 \rightarrow 1.3 \)
\( \frac{I}{I_0} = 0.5, \ A = 0.3 \)

If molecule opaque disk (\( r \sim 1 \text{ Å} \)), \( \varepsilon \sim 10^5 \text{ M}^{-1} \text{ cm}^{-1} \)

For good chromophore \( \varepsilon \sim 10^4 \), so 10% light absorbed

Absorption Bands characterized by \( \lambda_{\text{max}}, \epsilon_{\text{max}} \)
Chromophores in Biopolymers

\[ \lambda_{\text{max}} > 190 \text{ nm (abs. by } O_2) \]
\[ \lambda_{\text{max}} > 240 \text{ nm} \]
Proteins: Phe, Tyr, Try
Nucleic Acid: Bases (A, C, G, U, T)
Conjugated Polyenes, Hemes (Metal Ions)
“External” (Environmental) Factors Affecting Abs. Spec. ($\lambda_{\text{max}}, \varepsilon_{\text{max}}$)

Typical Physiological Conditions:
Solvent H$_2$O, pH 7.0, I = 0.15 M, T = 0-100°C

Effects on Monomers vs. Effects on Biopolymers
1. pH - Ionizable Functional Groups
2. Temp.
3. Solvent
4. Other Chromophores
Nucleic Acid Melting Curves

Equilibrium btwn single strands and duplex
\( \alpha = \) fraction of strands in duplex form

\[
K = \frac{[AB]}{[A][B]} = \frac{2\alpha}{(1 - \alpha)^2} C_T
\]

At each Temp.

\[
Abs. = \frac{C_T l}{2} \left[ (\epsilon_A + \epsilon_B)(1 - \alpha) + \epsilon_{AB} \alpha \right]
\]

At Melting Temp., \( \alpha = 0.5 \)

\[
\frac{1}{T_M} = \frac{R \ln(C_T/4)}{\Delta H^0} + \frac{\Delta S^0}{\Delta H^0}
\]
\[
\frac{1}{T_M} = \frac{R \ln(CT / 4)}{\Delta H^0} + \frac{\Delta S^0}{\Delta H^0}
\]