UV absorption of Amino Acids

Figure 13.15: Absorption spectra of the aromatic amino acids (tryptophan, tyrosine, and phenylalanine) at pH 6.

Figure 13.16: UV absorption spectra of three α-amino acids in aqueous solution at pH 6.

Polypeptide Spectra

The contribution of the amide linkages to the absorption spectra can be seen by comparing the spectrum of lysine hydrochloride in figure 13.16 with that of poly-L-lysine hydrochloride in the random-coil form (figure 13.17). The broad absorption centered at 192 nm ($\epsilon_{192} = 7100 \text{ M}^{-1} \text{ cm}^{-1}$) is characteristic of the amide linkages in poly-L-lysine and increases the absorbance in this region by about tenfold over that of the free amino acid. All proteins have contributions to the absorption spectra in the region around 190 nm (180 to 200 nm) from the polypeptide backbone; however, these are accompanied by absorption contributions from certain of the side chains, especially the aromatic ones.

Figure 13.17: UV-absorption spectra of poly-L-lysine hydrochloride in aqueous solution; random coil, pH 6.0, 25°C, helix, pH 10.4, 25°C, β-form, pH 10.4, 52°C.

UV absorption of Amino Acids

Note the Log scale

What is absorbance of a 0.01 M solution of phenylalanine if path is 1 cm

\[ A = \varepsilon cx \]

\[ A = 100 \times 0.01 \times 1 = 1 \]

Fraction transmitted = ? 0.1

What is absorbance of a 0.01 M solution of tyrosine if path is 1 cm

\[ A = 1000 \times 0.01 \times 1 = 10 \]

Fraction transmitted = ? 10^{-10}
**HOMEWORK #7**

CHMY 361
Nov. 18, 2013

**HANDOUT #10**

Due Fri., Nov. 22 in class

1. Given the information on the left hand plot, find the molar concentrations of tryptophan and compound X in the solution that gives the absorbance spectrum on the right. The path length is 0.10 cm. (Note: To have reasonable accuracy, use wavelengths with $A = 0.2$ or greater and extinction coeff. = 500 or greater to solve the problem. Realize that the problem cannot be solved if you pick two wavelengths for which the ratio of extinction coefficients is the same.) That will give you two identical equations, but you need two independent equations to solve for 2 unknowns. Therefore try to pick two wavelengths such that the ratio of extinction coefficients differs by a factor of 2 or greater.

![Molar Extinction Coefficient vs Wavelength](image1.png)

![Absorbance vs Wavelength](image2.png)

2. Assuming the absorption band at 240 nm for compound X is not due to the lowest excited electronic state, make a drawing of the fluorescence spectrum you expect if you excite the molecule with 240 nm light.
Using the Beer-Lambert Law to determine concentrations of a mixture of two absorbing species

\[
\begin{align*}
\text{Absorbance is additive: consider 2 absorbers } M & \text{ and } N \\
A_1 &= \varepsilon_1^M [M] + \varepsilon_1^N [N] \text{ at wavelength 1} \\
A_2 &= \varepsilon_2^M [M] + \varepsilon_2^N [N] \text{ at wavelength 2}
\end{align*}
\]

What if \( \varepsilon_1^M = \varepsilon_1^N \)?? (isosbestic point)

Then \( A_1 = \varepsilon [M] + \varepsilon [N] = \varepsilon ([M] + [N]) \)

In other words at isosbestic point you get the \text{TOTAL} concentration
Green is the unknown mixture of Trp and Tyr
Red is pure Tyr - Blue is pure Trp
Purple is 50-50 Trp + Tyr -

Example 13.1
What happens during absorption of light by Tryptophan?

Highest Occupied Molecular Orbital (a linear combination of atomic p orbitals)

Ground State

LUMO +1 , etc,
Lowest Unoccupied Molecular Orbital (electron excited)

Excited State (fluorescing state)

LUMO +1 , etc,

LUMO

Excited State  (fluorescing state)

Lowest Unoccupied Molecular Orbital (electron excited)
Fluorescence lifetime ~5 ns (exponential decay)

Similar to Fig. 13.5 of our Textbook.

\[ \Delta E = h\nu \]

HOT!!!

vibrational relaxation ~1 ps

internal conversion ~1 ps

\( S_2 \) (2nd excited state)

\( S_1 \) (1st excited state)

\( S_0 \) (ground state)

Vibrational levels

Absorption & Fluorescence

Fluorescence
What is fluorescence lifetime?

\[ \frac{d(\text{Intensity})}{dt} = -k \ (\text{Intensity}) \]

Fluor. intensity at time \( t \) = (Fluor. Intensity at time 0) \( \times e^{-kt} \)

\[ = (\text{Fluor. Intensity at time 0} \times e^{-t/\tau}) \]

\( \tau = \frac{1}{k} \)

\( \tau = \text{inverse of 1st order rate constant} \)

**Fluorescence “Quenching”**

A reaction with another molecule that competes with the rate of fluorescence
Highest Occupied Molecular Orbital (a linear combination of atomic p orbitals)
Lowest Unoccupied Molecular Orbital (electron excited)

Excited State (fluorescing state)

Lowest Unoccupied Molecular Orbital (electron excited)
Iodide ion collides; (has higher HOMO) will quench fluorescence
Electron transfer from I⁻ to indole makes a radical pair that cannot fluoresce. (would violate Pauli exclusion)

Radical Pair (can’t fluoresce)

Electron transferred from iodide to vacancy in HOMO of ring i.e., QUENCHING
Phosphorescence

$S_1$
(1st excited state)

$S_0$
(ground state)

Intersystem crossing, ns

Lowest triplet state
(unpaired spins)

Fluorescence:
~ 5 ns

Phosphorescence
Slow, forbidden ($10^{-6}$ to 10 seconds)

Requires rigid, oxygen free environment

$S_1$ abs
$S_1$ fluor

Phos

Wavelength →