Today’s class:

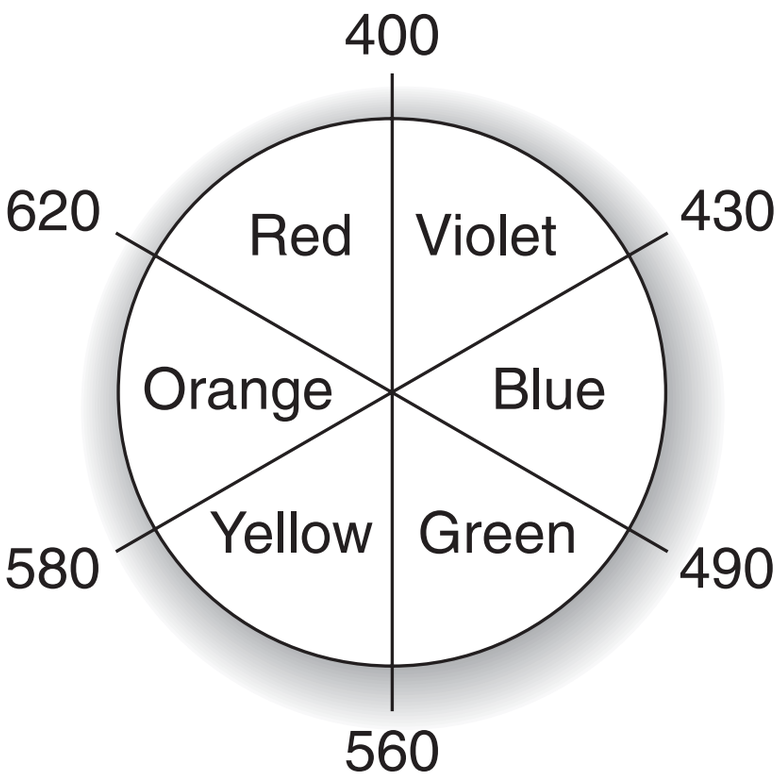
Absorption Spectroscopy

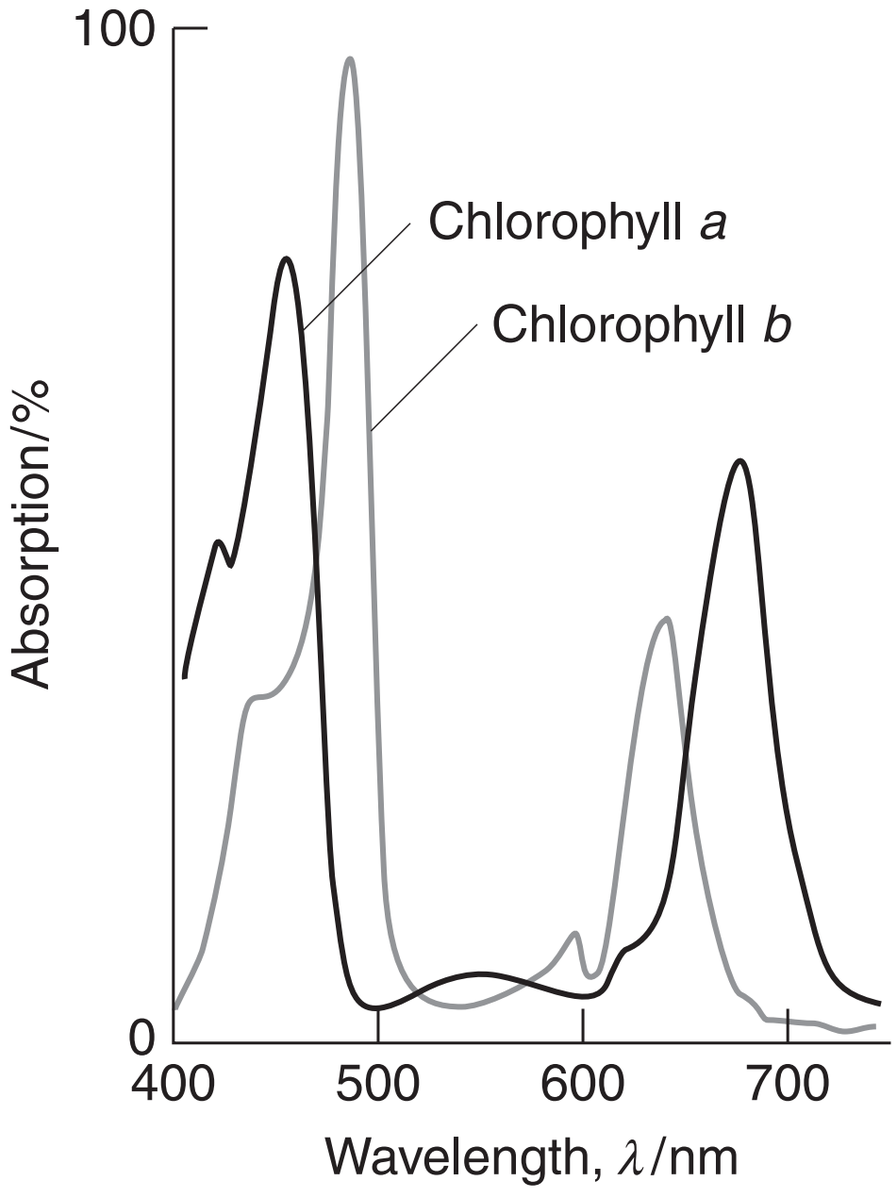
This lecture follows the materials from the following books

- *Spectroscopy for the Biological Sciences, by GG Hammes, Wiley, 2005 - Chapter 3*
- *Physical Chemistry for Life Sciences, by PW Atkins and JD Paula, Oxford, 2006*

The ultraviolet and visible spectra

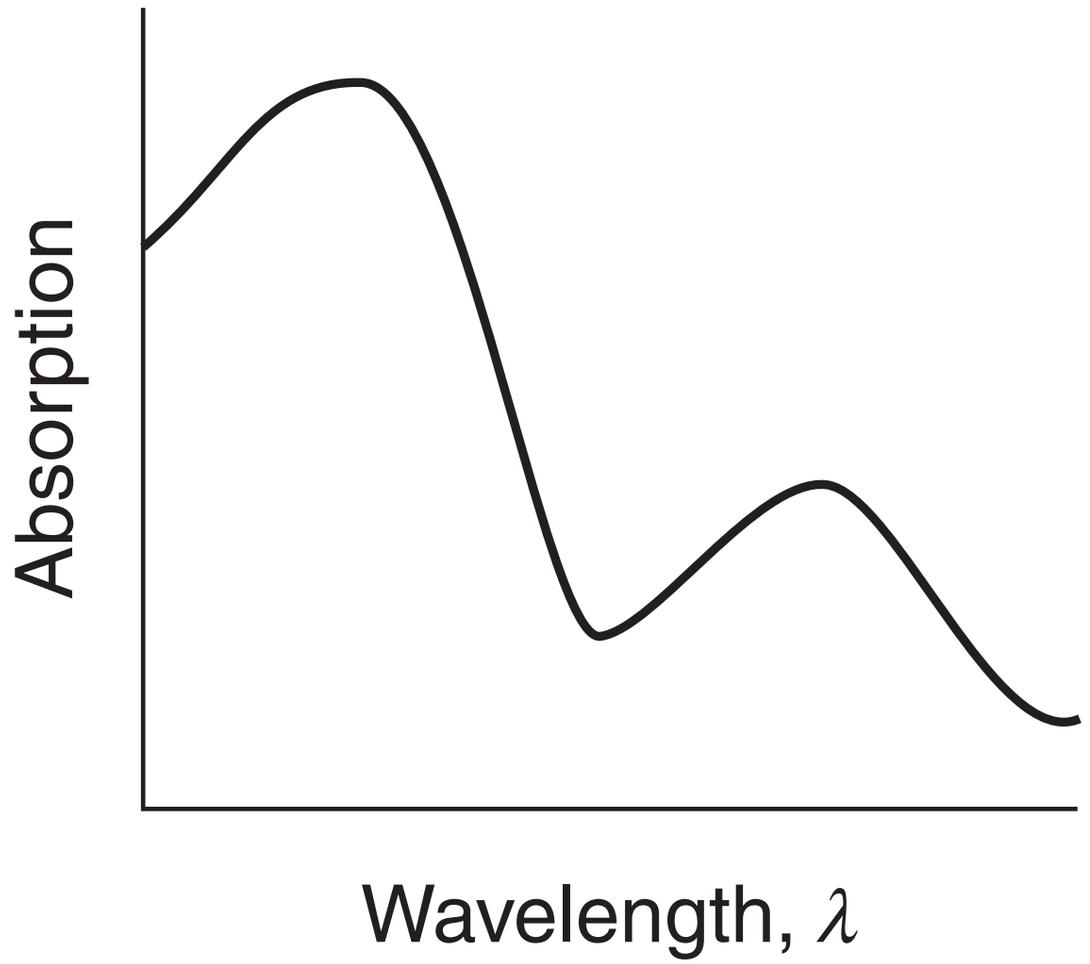
An artist's color wheel



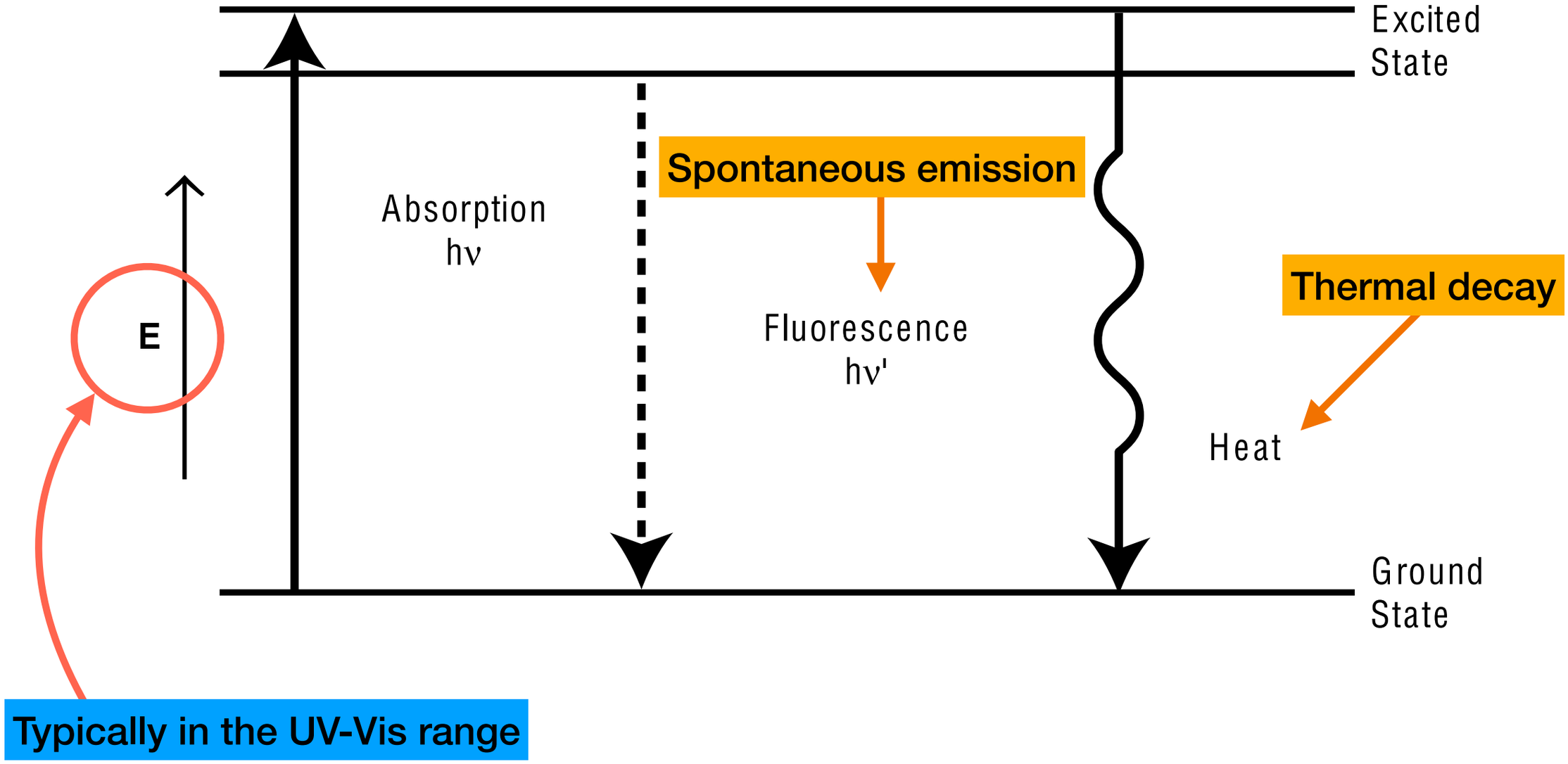


Absorption spectra may not always predict the colors

This absorption band has peaks but also long tails that makes it hard to predict colors



Electronic excitation in molecules

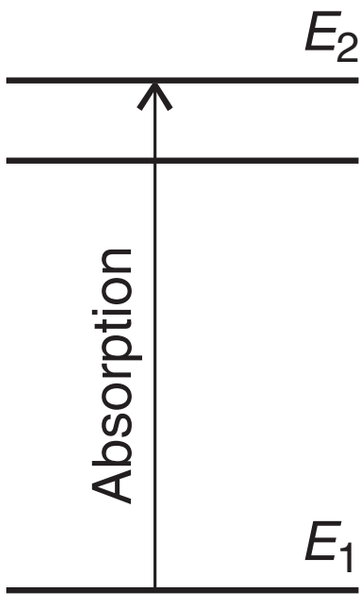


How long does it take to absorb?

Timescale of electronic excitation

For any quantum mechanical transition with a time-scale τ:

$$\tau \Delta E \sim \hbar$$



$$\Delta E = |E_1 - E_2| = h\nu$$ Here, ν = Bohr frequency

This relation often written with wavelength of light, $\lambda = \dfrac{c}{\nu}$

Or with the wavenumber, $\bar{\nu} = \dfrac{\nu}{c} = \dfrac{1}{\lambda}$ **Unit of $\bar{\nu} = \mathrm{cm}^{-1}$**

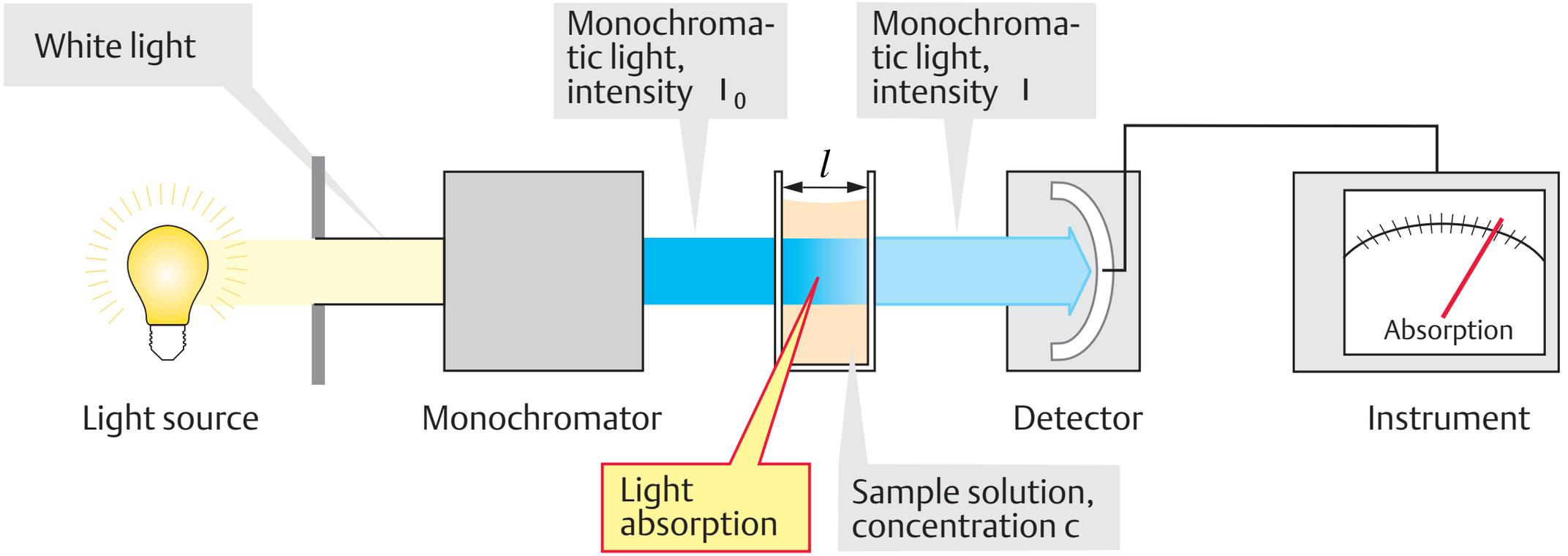
$$\implies \tau \sim \frac{\hbar}{\Delta E} = \frac{\hbar\lambda}{hc} = \frac{\lambda}{2\pi c} = \frac{1}{2\pi c\bar{\nu}}$$

For UV-VIS, $\lambda \sim 100 - 800$ nm

For $\lambda = 500$ nm, $\bar{\nu} = 20000\ \mathrm{cm}^{-1}$ $\implies \tau \approx 0.3$ fs

Typical timescales of electronic excitations are ~ femtoseconds

How much light is absorbed by the sample?



Beer-Lambert law

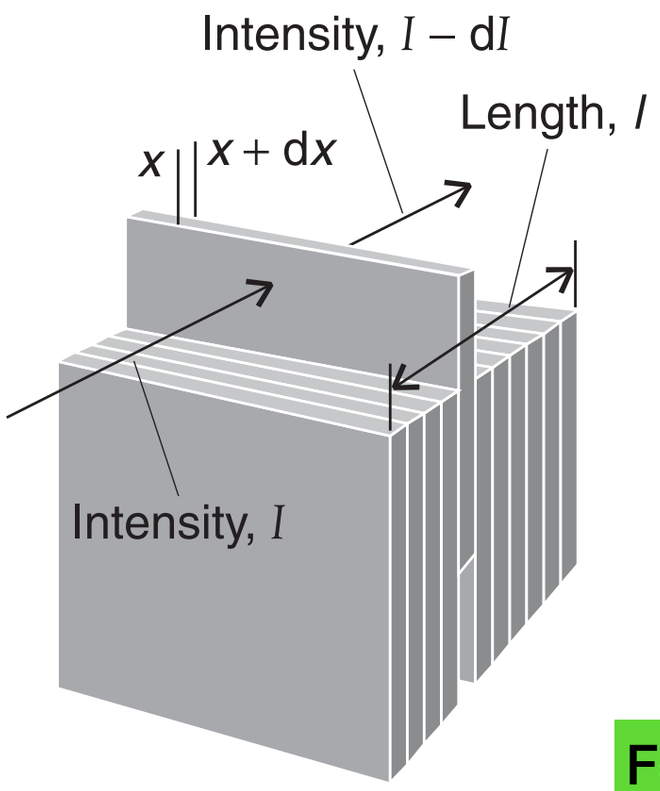
The measured absorbance for a single compound is directly proportional to the concentration of the compound and the length of the light path through the sample

Measured absorbance or optical density is defined as

$$A = log\left(\frac{I_0}{I}\right) \qquad \propto c \quad \text{Beer law}$$

$$\propto l \quad \text{Lambert law}$$

Understanding the Beer-Lambert law



Decrease in intensity of light through sample

$$dI \propto c \times I \times dx$$

$$\Longrightarrow \quad dI = -\kappa c I dx$$

$$\Longrightarrow \quad \frac{dI}{I} = -\kappa c dx$$

This is for one slice of sample

For the entire sample thickness

$$\Longrightarrow \quad \int_{I_0}^{I} \frac{dI}{I} = -\kappa c \int_{0}^{l} dx \qquad \Longrightarrow \quad ln\left(\frac{I}{I_0}\right) = -\kappa c l$$

This can be rearranged to

$$\Longrightarrow \quad \log\left(\frac{I_0}{I}\right) = (\kappa \; ln10) \; cl \qquad \Longrightarrow \quad \boxed{A = \epsilon c l}$$

Final form of Beer-Lambert law

where, $\epsilon = \dfrac{\kappa}{ln10}$

molar extinction coefficient

Alternate versions of the Beer-Lambert law

$$A = log\left(\frac{I_0}{I}\right) = \epsilon cl$$

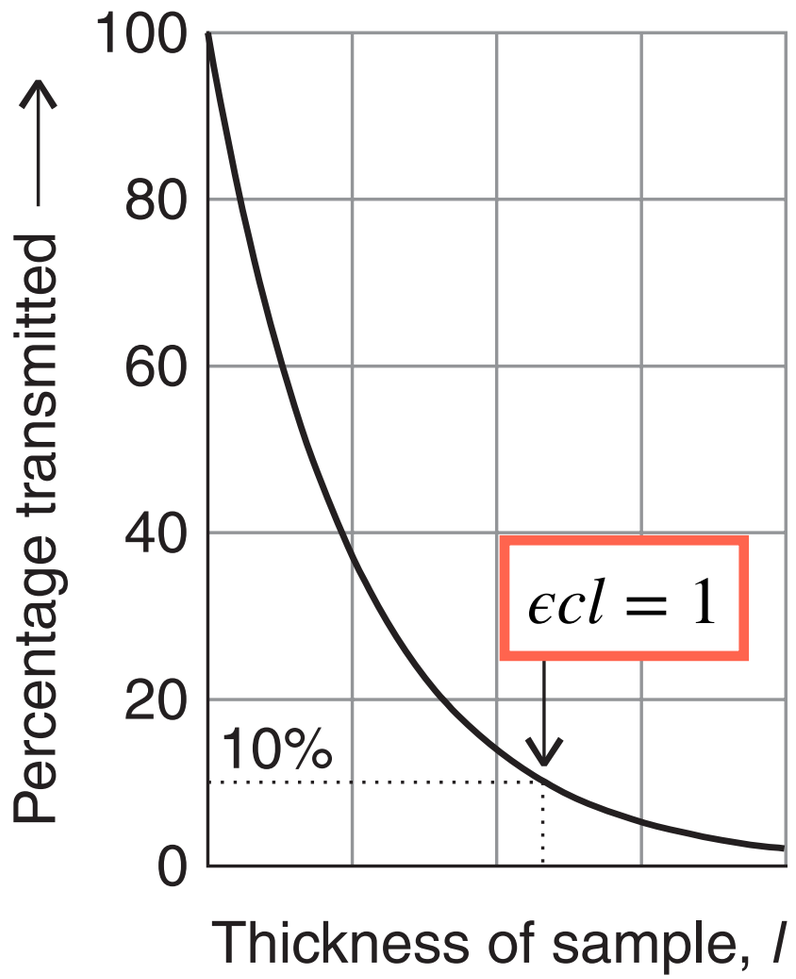
This is often written as

$$A = -\,log\left(\frac{I}{I_0}\right) = -\,log\ T = \epsilon cl$$

Where T = transmittance of the sample $= \frac{I}{I_0}$

$$log\left(\frac{I}{I_0}\right) = -\,\epsilon cl$$

$$\Longrightarrow\ I = I_0\ 10^{-\epsilon cl}$$



Units of molar extinction coefficient

$$A = \epsilon c l$$

Dimensionless

$$[\epsilon] = \frac{[A]}{[c][l]}$$

$$[\epsilon] = \frac{1}{[ML^{-3}][L]}$$

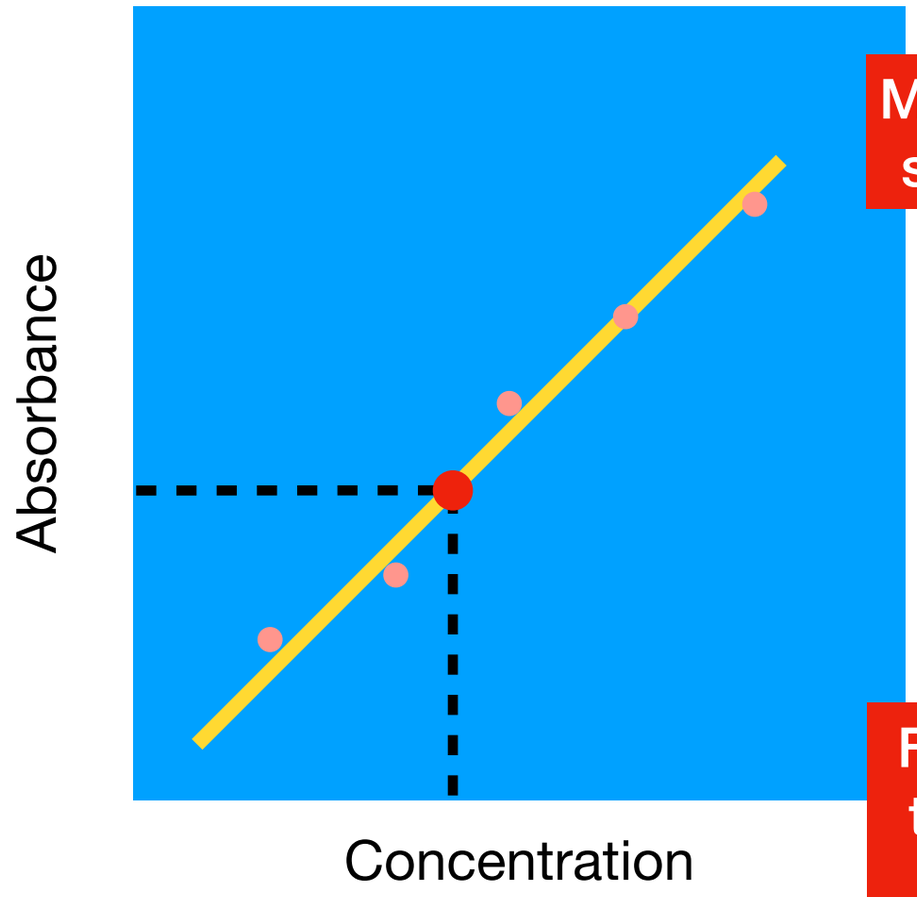
Where $c \sim \mathrm{M} \sim \mathrm{moles/L}$, and path length, $l \sim \mathrm{cm}$

Then unit of $\epsilon \sim \mathrm{L\ mol^{-1}\ cm^{-1}}$

Sometimes also used unit of $\epsilon \sim \mathrm{M^{-1}cm^{-1}}$ **Here, M = molarity**

ϵ is also called molar absorption coefficient

Determination of molar extinction coefficient



Measured at a wavelength where sample absorption is maximum

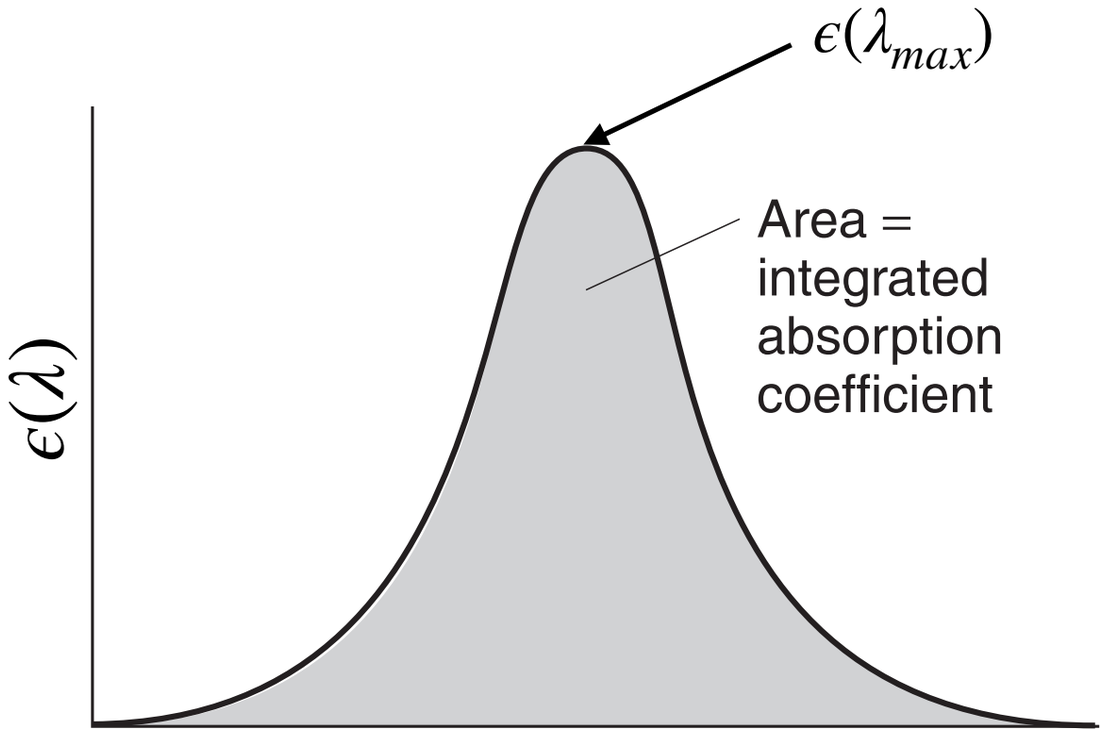
Such plots are called calibration curves

For polychromatic light sources there is deviation from linearity because ϵ varies with λ

From such a plot we can calculate two things

- Molar extinction coefficient from the slope
- Any unknown concentration of the absorbing species

The molar extinction coefficient depends of wavelength



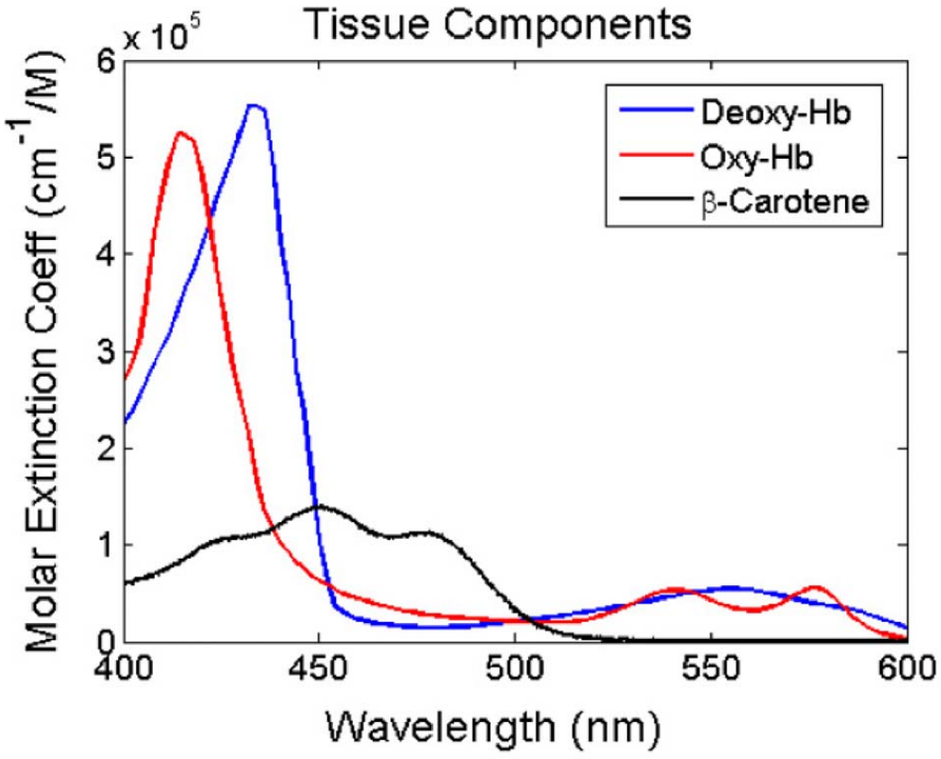


Figure 2. Dominant absorbers of breast tissue in the UV-visible spectrum. Molar extinction coefficient of oxy- and deoxy- hemoglobin and β-carotene in the 400–600 nm range.

Lo et al Plos One 2013

λ_{max} = wavelength at which absorption is maximum

Experimentally we use: $\epsilon(\lambda_{max})$ is the dependence is sharp

Alternatively, an integrated absorption coefficient can also be used

$$\mathscr{A} = \int_{\lambda} \epsilon(\lambda)$$

The Beer-Lambert law in action: monitoring concentration

From Beer-Lambert law, $$c = \frac{A}{\epsilon l}$$

Concentration can be determined for species with known ϵ

Radiation of wavelength 280 nm passed through 1.0 mm of a sample containing an aqueous solution of the amino acid tryptophan and the measured absorbance was A = 0.27.

$$\epsilon_{tryp}(280\ \text{nm}) = 5.4 \times 10^3\ \text{L mol}^{-1}\ \text{cm}^{-1}$$

$$\begin{aligned} c &= \frac{0.27}{(5.4 \times 10^3\ \text{L mol}^{-1}\ \text{cm}^{-1}) \times (0.10\ \text{cm})} \\ &= 5.0 \times 10^{-4}\ \text{mol L}^{-1} = 0.50\ \text{mmol L}^{-1} \end{aligned}$$

The Beer-Lambert law can be used to measure protein conc

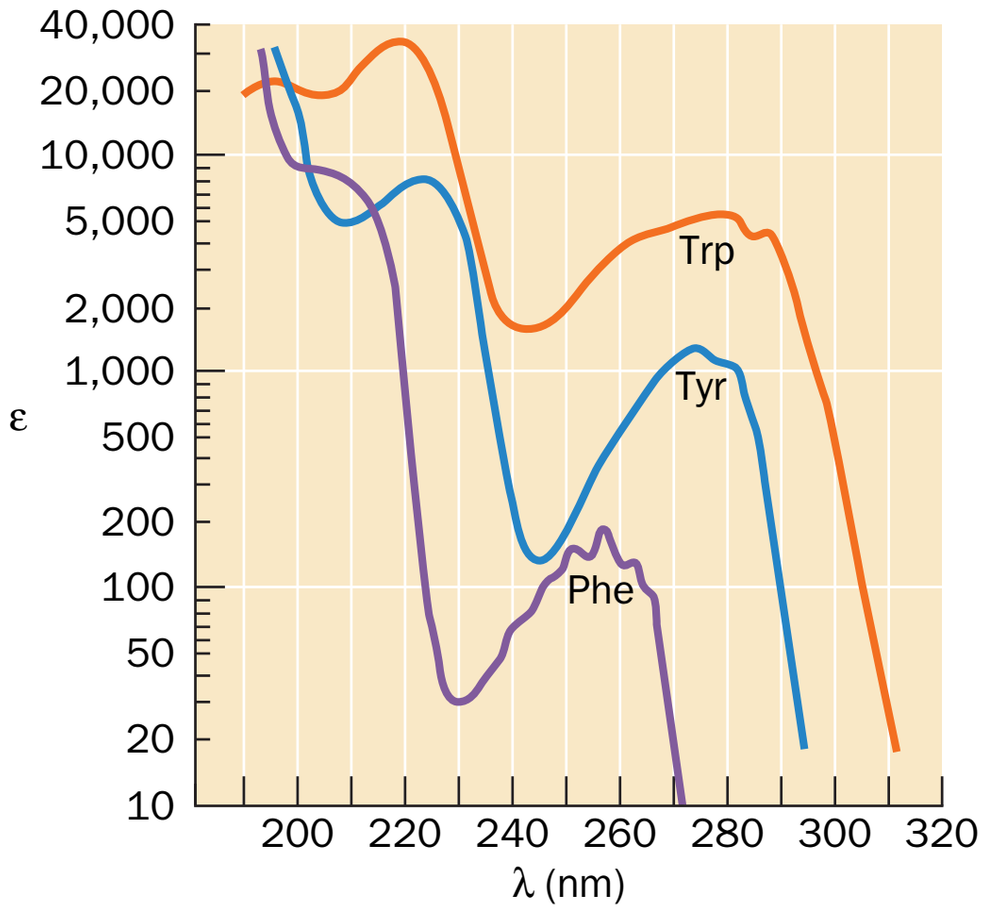


FIG. 5-4 UV absorbance spectra of phenylalanine, tryptophan, and tyrosine. Here the **molar absorptivity** (ε when c is expressed in mol · L^{-1}) for each aromatic amino acid is displayed on a log scale. [After Wetlaufer, D.B., *Adv. Prot. Chem.* **7,** 310 (1962).]

- Protein absorbance depends on the amino acid composition as they primarily absorb strongly in UV region
- Absorbance of a protein in range 200-400 nm can be converted into conc of a protein
- However, some **chromophores** in proteins can be responsible for the color as they absorb in visible region (> 400 nm)

Chromophores: parts of molecule responsible for color, like aromatic rings, double bonds, carbonyl groups

Electronic absorption properties of amino acids and bases

In water and at pH = 7

Compound	λ_{max}/nm	ε_{max}/(10^3 L mol^{-1} cm^{-1})
Tryptophan	280	5.6
Tyrosine	274	1.4
Phenylalanine	257	0.2
Adenine	260	13.4
Guanine	275	8.1
Cytosine	267	6.1
Uracil	260	9.5