

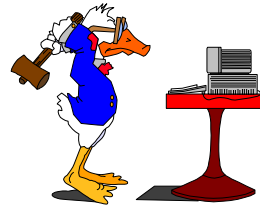
Pharmacy 325

UV-Visible Light Spectroscopy

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Hours: anytime after 4 pm

Lecture Notes Available At:

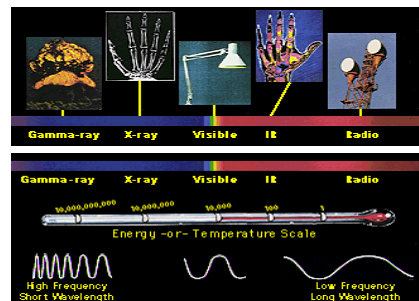
- <http://redpoll.pharmacy.ualberta.ca>
- <http://www.pharmacy.ualberta.ca/pharm325/>



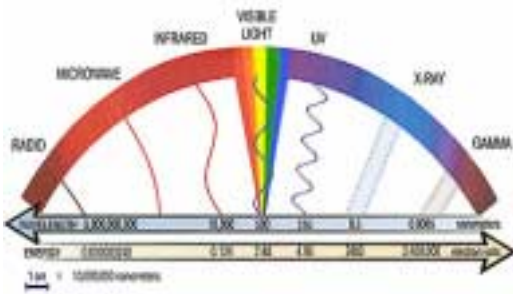
Definition

- **Spectroscopy** - The study of the interaction of electromagnetic radiation with matter

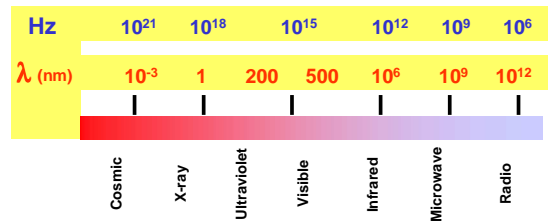
Electromagnetic Spectrum



Electromagnetic Spectrum



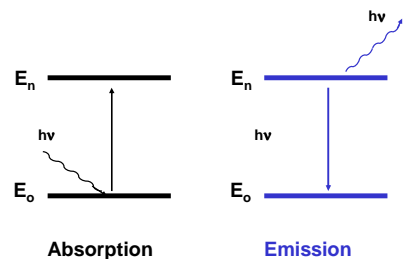
Electromagnetic Spectrum



Type of EM Interactions

- **Absorption** - EM energy transferred to absorbing molecule (transition from low energy to high energy state)
- **Emission** - EM energy transferred from emitting molecule to space (transition from high energy to low energy state)
- **Scattering** - redirection of light with no energy transfer

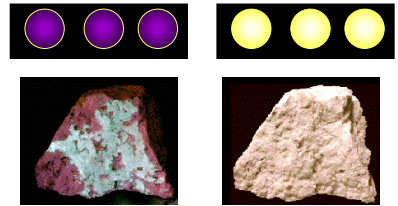
Absorption vs. Emission



Absorption



Fluorescence (Emission)



Definitions

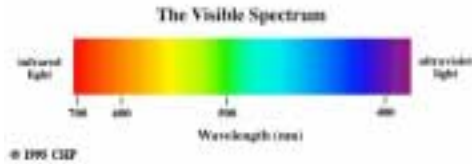
- E = energy (Joules, ergs)
- c = speed of light (constant)
- λ = wavelength
- h = Planck's constant
- ν = "nu" = frequency (Hz)
- $\text{nm} = 10^{-9} \text{ m}$
- $\text{\AA} = \text{angstrom} = 10^{-10} \text{ m}$

Key Formulae

- $E = h\nu$
- $h = 6.626 \times 10^{-34} \text{ J-s}$
- ν = frequency in Hz, E = energy

- $\lambda = c/\nu$
- $c = 3.0 \times 10^8 \text{ m/s}$
- λ = wavelength, ν = frequency in Hz

Visible Light



Visible Light

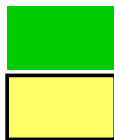
	Red	R	700 nm
	Orange	O	650 nm
	Yellow	Y	600 nm
	Green	G	550 nm
	Blue	B	500 nm
	Indigo	I	450 nm
	Violet	V	400 nm

Complementary Colours

Absorbed



Observed



Types of Light Spectroscopy

- **Absorption spectroscopy**
 - Measures amount of light absorbed
 - Most common, non-destructive
 - Concentration, pH measures, purity, ID
- **Atomic emission spectroscopy**
 - Measures light emitted from burned sub.
 - Elemental analysis
- **Luminescence (Luminol)**

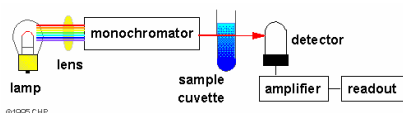
Types of Light Spectroscopy

- **Fluorescence spectroscopy**
 - Things fluoresce when they emit at higher λ than what they absorb
 - Measure solvent interactions, distances
- **Circular Dichroism spectroscopy**
 - Absorption of circular polarized light
 - Chiral compound identification
- **Transmission spect. (colorimetry)**

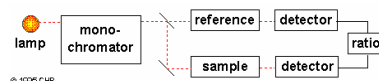
UV vs. IR vs. NMR

- UV has broad peaks relative to IR & NMR
- UV has less information than IR & NMR
- UV spectra are easier to collect
- UV spectra are faster to collect
- UV spectrometers are cheaper
- UV spectra require only nanograms of material or chemicals

Single Beam Spectrophotometer

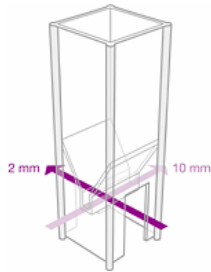


Dual Beam Spectrophotometer



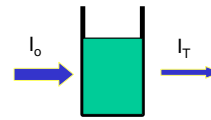
Cuvettes (sample holder)

- Polystyrene
 - 340-800 nm
- Methacrylate
 - 280-800 nm
- Glass
 - 350-1000 nm
- Suprasil Quartz
 - 160-2500 nm

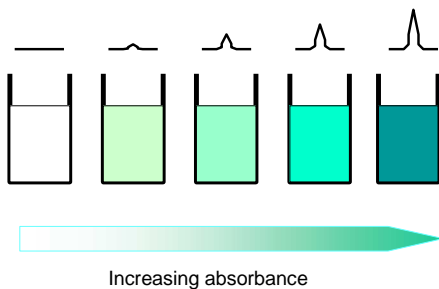


Definitions

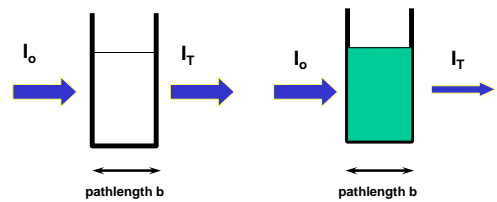
- I_o = intensity of light through blank
- I_T = intensity of light through sample
- Absorption = $I_o - I_T$
- Transmittance = I_T/I_o
- Absorbance = $\log(I_o/I_T)$



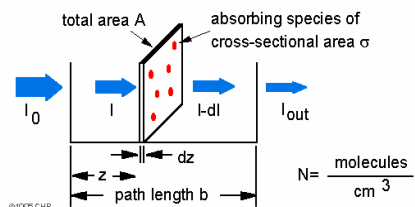
Absorbance & Beer's Law



Beer's Law




Beer's Law

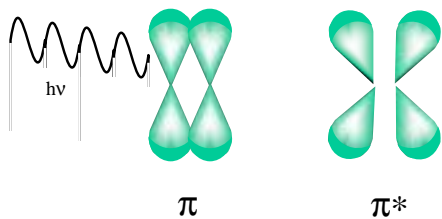


$$\text{Absorbance} = C\epsilon l$$

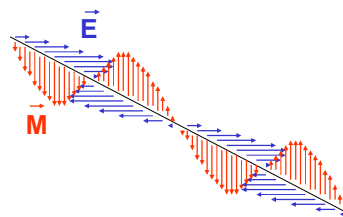
UV Activity

- Needs chromophores
- $C=C$, $C=O$, $N=N$, NO_2 , 
- $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions
- naphthoquinones, anthocyanins
- Non-absorbers...
 - long chain aliphatics
 - alcohols, ethers, non-conjugated mols.

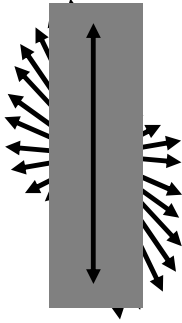
UV Activity



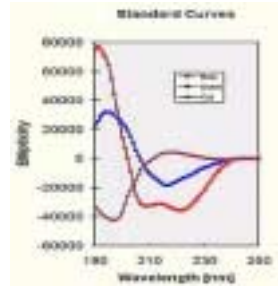
EM Radiation



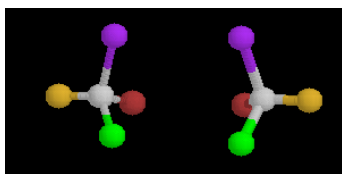
Circular Polarization



Circular Dichroism



CD can distinguish chirality



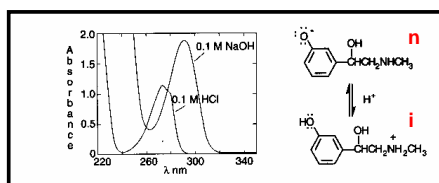
L-isomer

D-isomer

Practical Applications

- **Pharmacy Practice**
 - Ultraquin (psoriasis med. Needs UV. Act.)
 - Pregnancy tests (colorimetric assays)
 - Blood glucose tests, Bilichek
 - ELISA's
- **Pharmaceutics**
 - pH titrations, purity measurement
 - concentration measurement

pKa Measurement with UV



Titration of Phenylephrine

$$\text{pKa} = \text{pH} + \log \frac{A_i - A}{A - A_n}$$

Practical Applications

- **Medicinal Chemistry**
 - compound ID (steroids, nucleosides)
 - monitoring isomerization, chirality
- **Pharmaceutical Biotechnology**
 - concentration/purity measurements
 - monitoring conformation of protein drugs
- **Pharmacokinetics/Med. Chem.**
 - HPLC monitoring and purification

Pharmaceutical Apps.

- On Line Analysis of Vitamin A and Coloring Dyes for the Pharmaceutical Industry
- Determination of Urinary Total Protein Output
- Analysis of total barbiturates
- Comparison of two physical light blocking agents for sunscreen lotions
- Determination of acetylsalicylic acid in aspirin using Total Fluorescence Spectroscopy
- Automated determination of the uniformity of dosage in Quinine Sulfate tablets using a Fibre Optics Autosampler
- Determining Cytochrome P450 by UV-Vis Spectrophotometry
- Light Transmittance of Plastic Pharmaceutical Containers