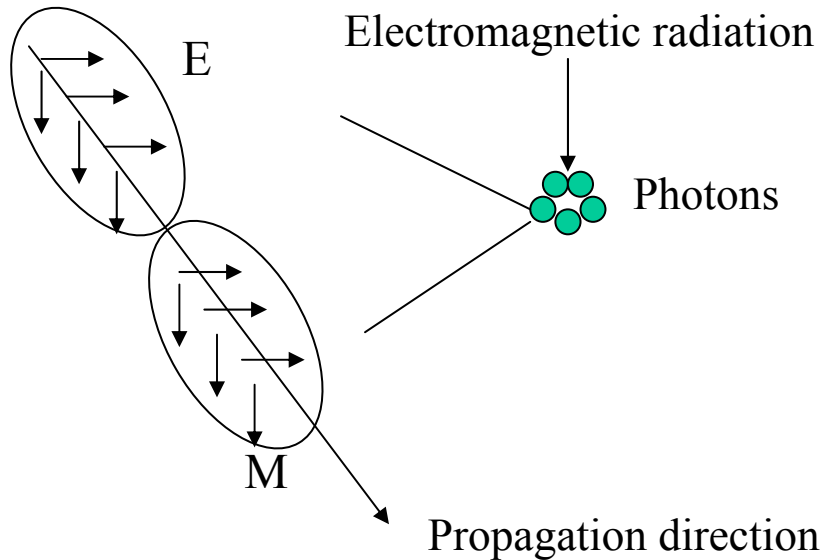


Spectrophotometry – Fundamentals

1. Electromagnetic radiation – properties



Relationships

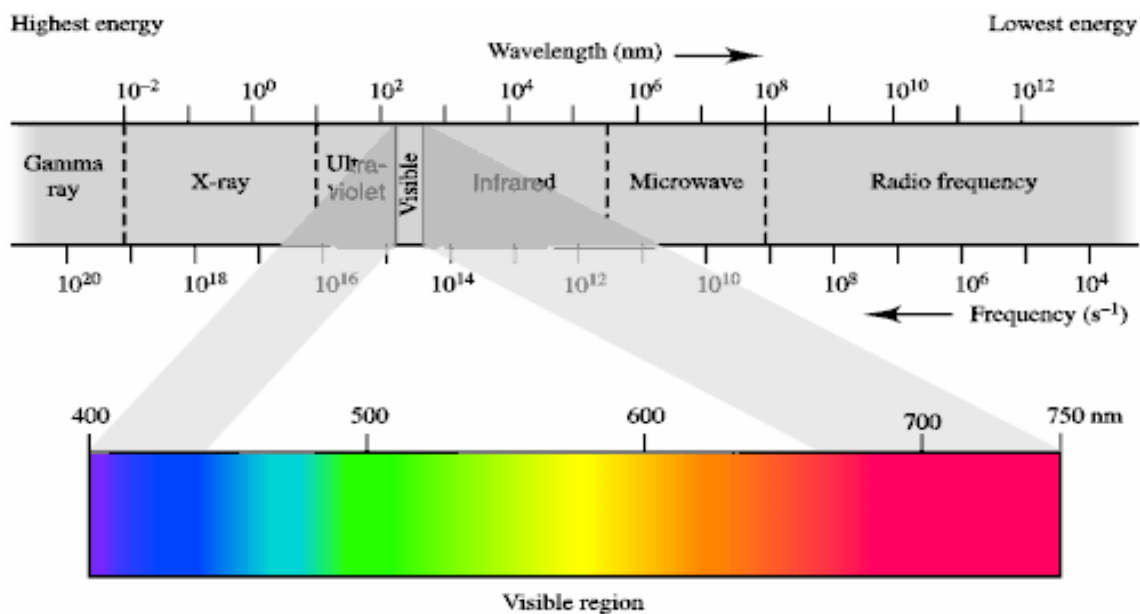
a. Frequency/Wavelength - $\nu\lambda = c$
where c = the speed of light in vacuum (2.998×10^8 m/s)

b. Energy/Frequency – $E = h\nu$
where h = Planck's constant (6.626×10^{-34} J s⁻¹), ν (Hz)

$$E = \frac{hc}{\lambda} = hc\bar{\nu}$$

where $\bar{\nu} = \frac{1}{\lambda}$ and called the wavenumber

2. Electromagnetic Spectrum



Why is a red solution red?

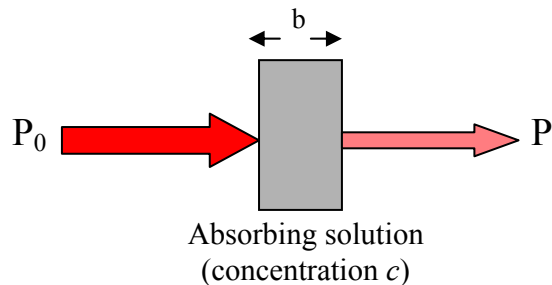
e.g. FeSCN^{2+}

Wavelength (nm)	Color	Complementary color
400-435	Violet	Yellow-green
435-480	Blue	Yellow
480-490	Green-blue	Orange
490-500	Blue-green	Red
500-560	Green	Purple

3. Absorption of Light

<u>Term & Symbol*</u>	<u>Definition</u>	<u>Alt. Name & Symbol</u>
Radiant power P, P_0	Energy of radiation	Radiation intensity I, I_0
Absorbance A	$\text{Log } P_0/P$	Optical density D
Transmittance T	P/ P_0	Transmission T
Path length of radiation b	-----	l, d
Absorptivity a	A/bc	Extinction coefficient k
Molar absorptivity ϵ	A/bc	Molar extinction coefficient

* Recommended by American Chemical Society



$$\text{Transmittance } T = P / P_0$$

$$\text{Absorbance } A = \log P_0 / P = -\log T = abc$$

↓
Beer's Law

%T	A
100	0.00
90	0.05
80	0.10
70	0.15
60	0.20
50	0.25
40	0.30
	0.35
	0.40
	0.45
30	0.50
	0.60
20	0.70
	0.80
10	1.00
	1.50

a. Beer's Law

$$A = abc_{(\text{g L}^{-1})} \text{ or } A = \epsilon bc_{(\text{mol L}^{-1})}$$

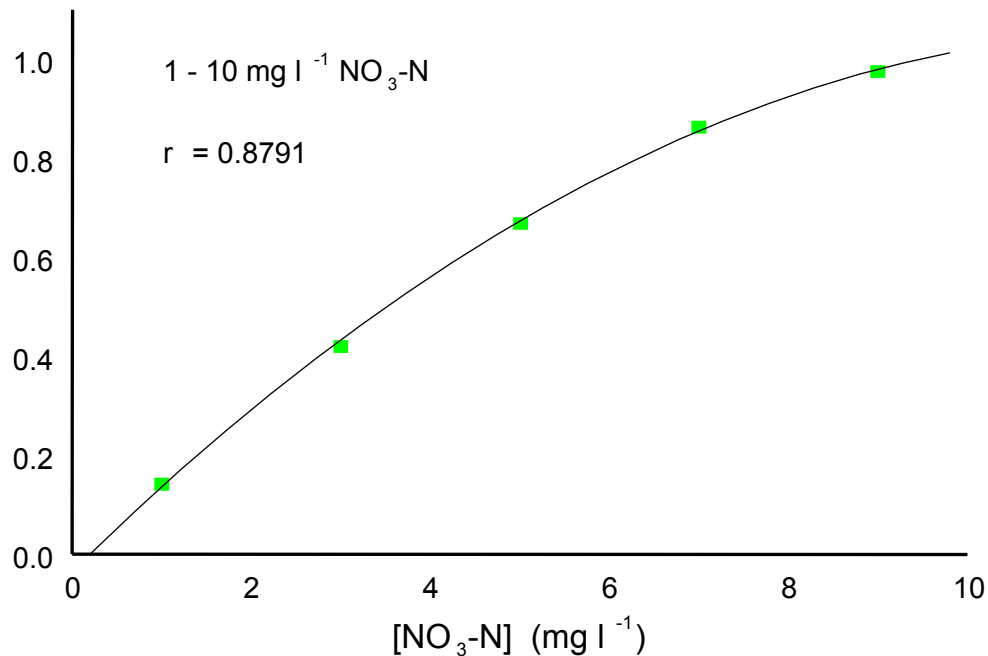
Ex: A 7.50×10^{-5} M solution of KMnO_4 has T of 36.4% when measured in a 1.05-cm cell at 525nm.

(1) Calculate the absorbance of this solution:

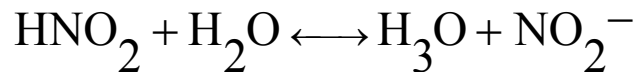
(2) Calculate the molar absorptivity of KMnO_4 :

b. Limitations to Beer's Law

(1). Real Limitations – high concentrated solutions, concentrated electrolyte solutions (proximity alters molecular absorption).



(2) Chemical Limitations – absorbing species participate in association or dissociation reactions, e.g. weak acids in concentrated solutions, complexation.



(3) Instrumental Deviations – polychromatic radiation used to measure absorbance, stray light.

- Use of filters, diffraction gratings
- Molar absorptivities must be equal

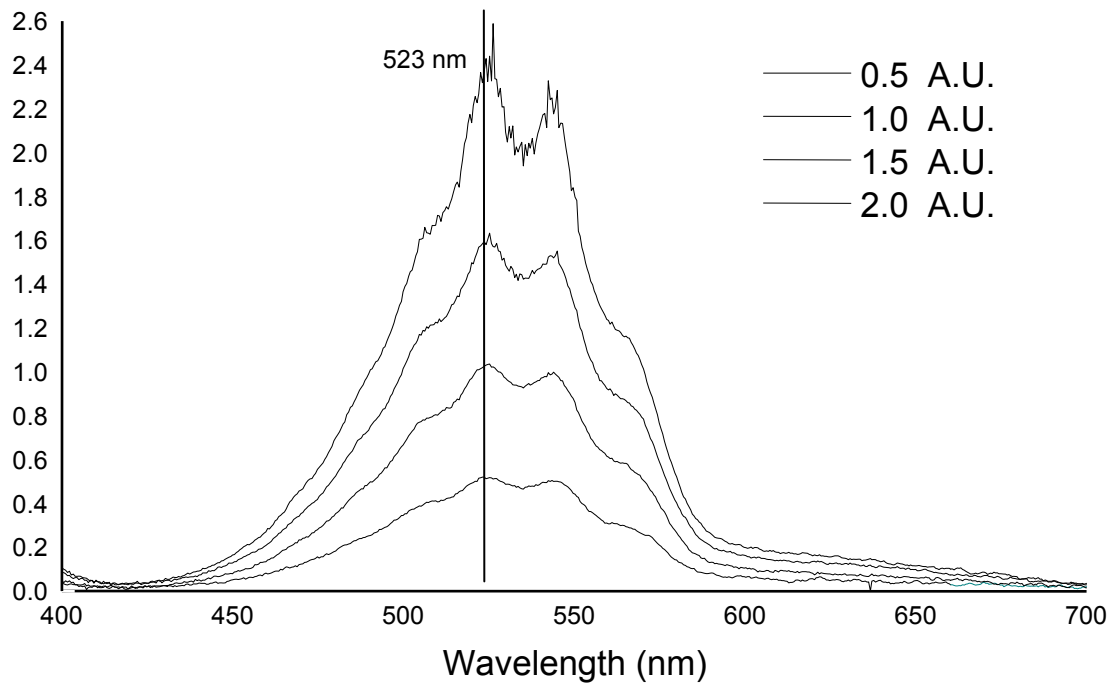
Stray radiation

$$A = \log \frac{P_0}{P}$$

$$A = \log \frac{P_0 + P_s}{P + P_s}$$

where P_s = incident power of stray radiation

4. Absorption Spectra



- Plotting of spectral data
- λ maximum, nm
- Solutions of different concentrations

5. Theory of Molecular Absorption

-every molecular species has a unique set of energy states; the lowest termed the *ground state*.

Excited State – energy of photon transferred to molecule:



a. Types of Molecular Transitions

- (1) Electronic transition – electron between two orbitals (UV-vis).

$$h\nu = \text{Energy Difference of Orbitals}$$

- (2) Vibrational transitions – vibrational states associated with bonds holding molecule. Involve simultaneous:

- (3) Rotational transitions – changes in rotational states, about its center of gravity.

Types of electronic transitions

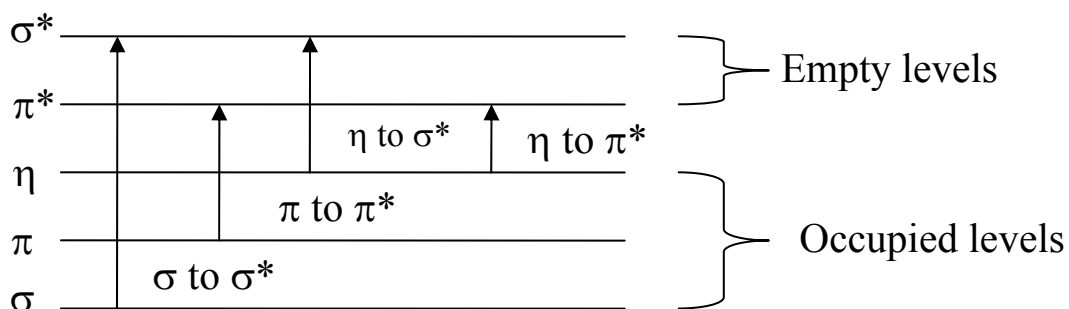
σ to σ^* \longrightarrow Alkanes

σ to π^* \longrightarrow Carbonyl compounds

π to π^* \longrightarrow Alkenes, carbonyl compounds, alkynes, azo compounds

η to σ^* \longrightarrow Oxygen, nitrogen, sulfur and halogen compounds

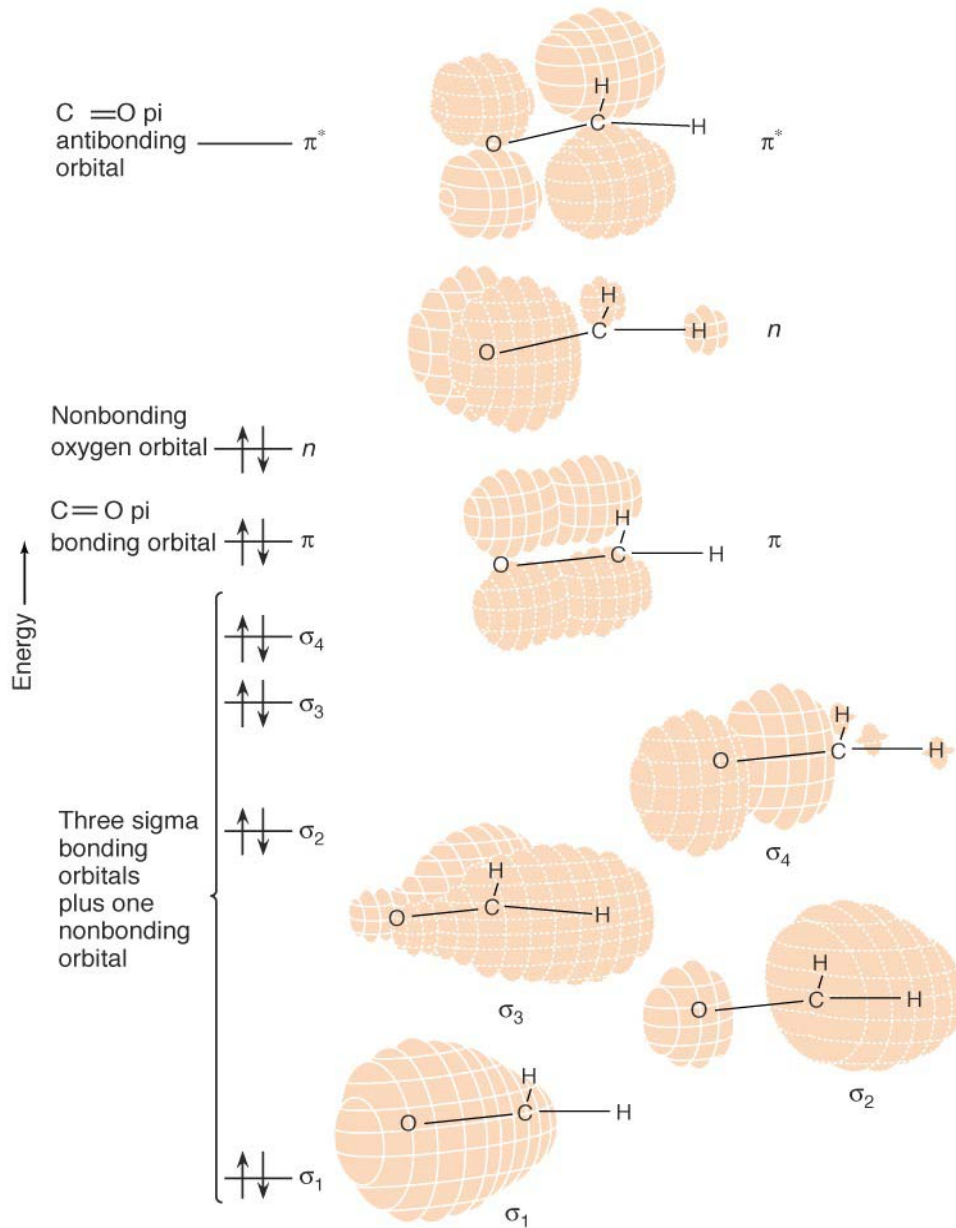
η to π^* \longrightarrow Carbonyl compounds



Highest occupied molecular orbital (HOMO)

Lowest unoccupied molecular orbital (LUMO)

$$E = h \nu = h \nu / \lambda = E(\text{LUMO}) - E(\text{HOMO})$$

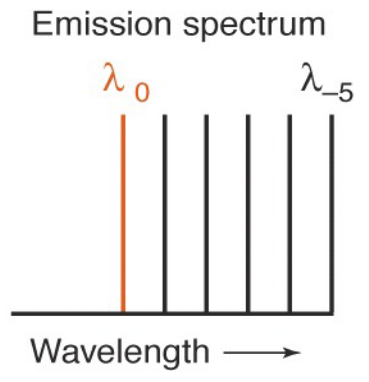
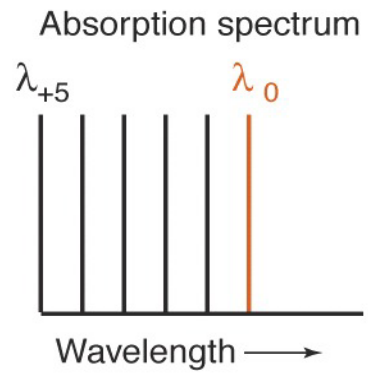
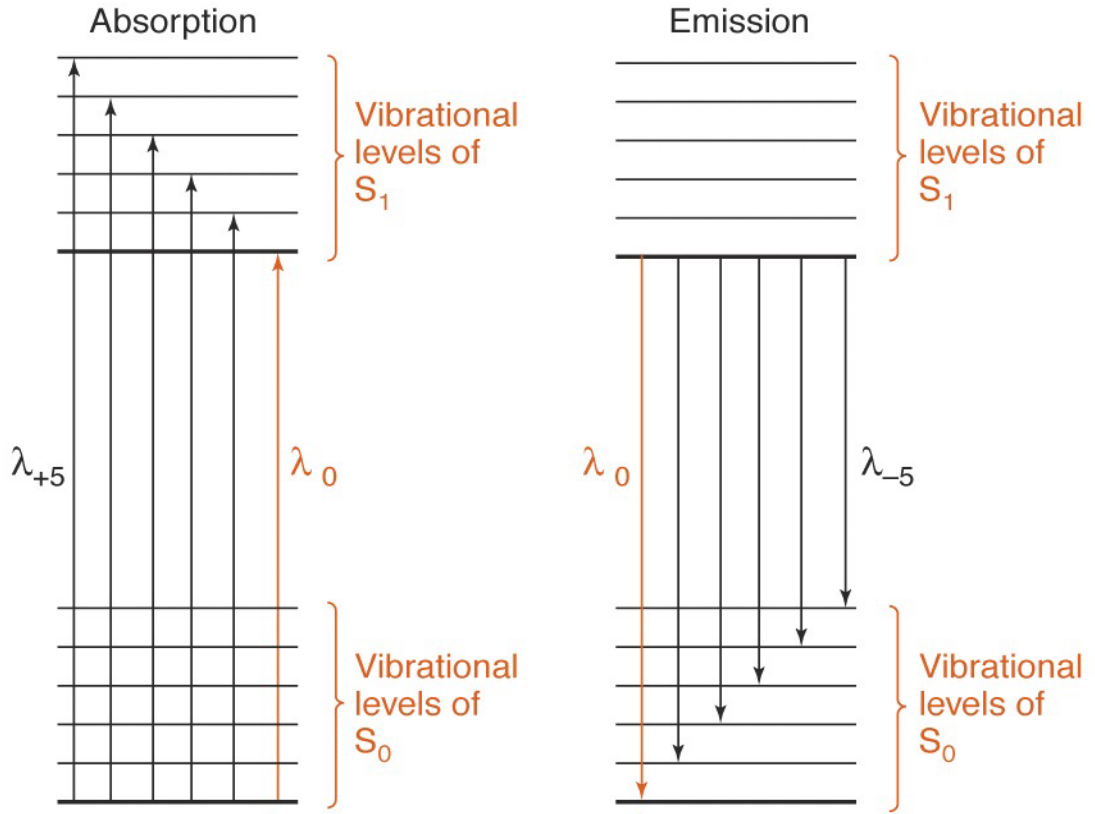


Therefore - Overall change in Energy with an orbital of a molecule is:

$$E = E_{\text{electronic}} + E_{\text{vibrational}} + E_{\text{rotational}}$$

$$E_{\text{electronic}} \approx 10 E_{\text{vibrational}}$$

$$\approx 100 E_{\text{rotational}}$$



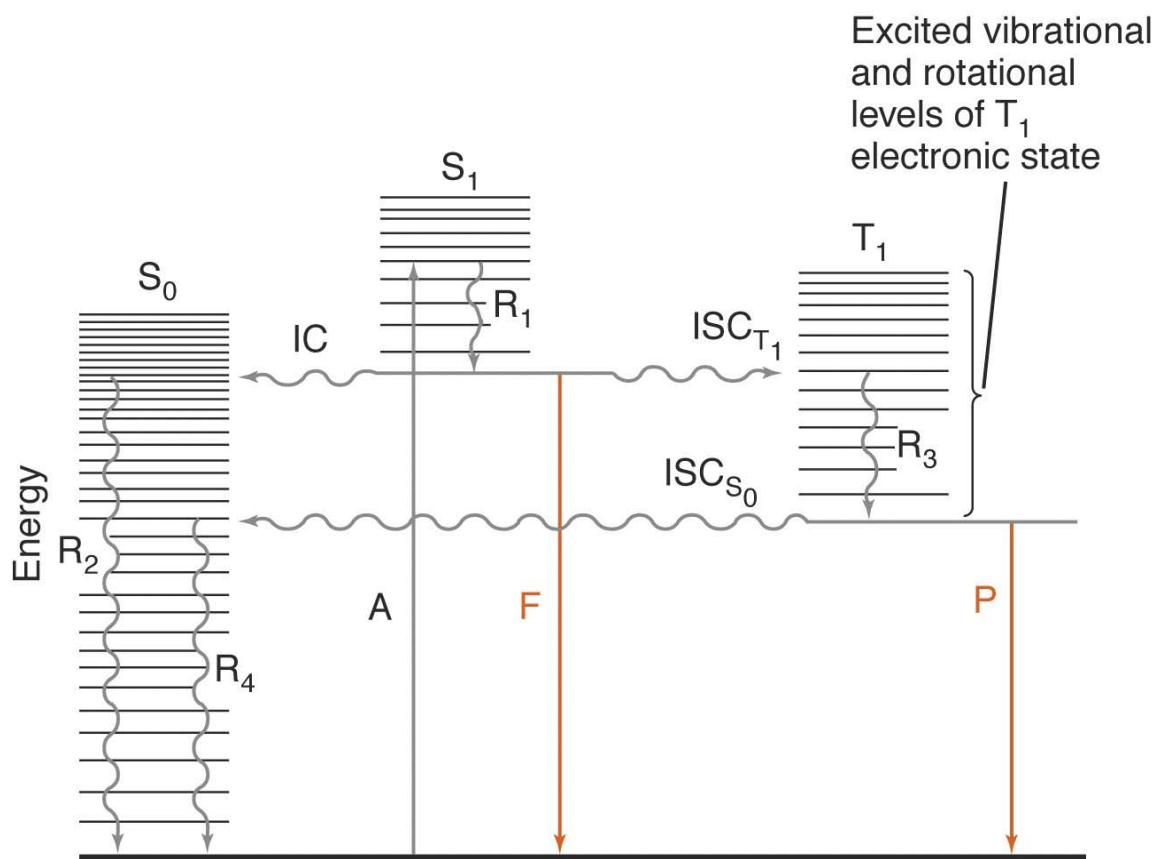
Emission Processes

b. Luminescence – emission of light from any excited state of a molecule.

- more sensitive than absorption measurements

(1) Fluorescence – emission of photon during transition between states with same spin quantum #'s (e.g. $S_1 \rightarrow S_0$)

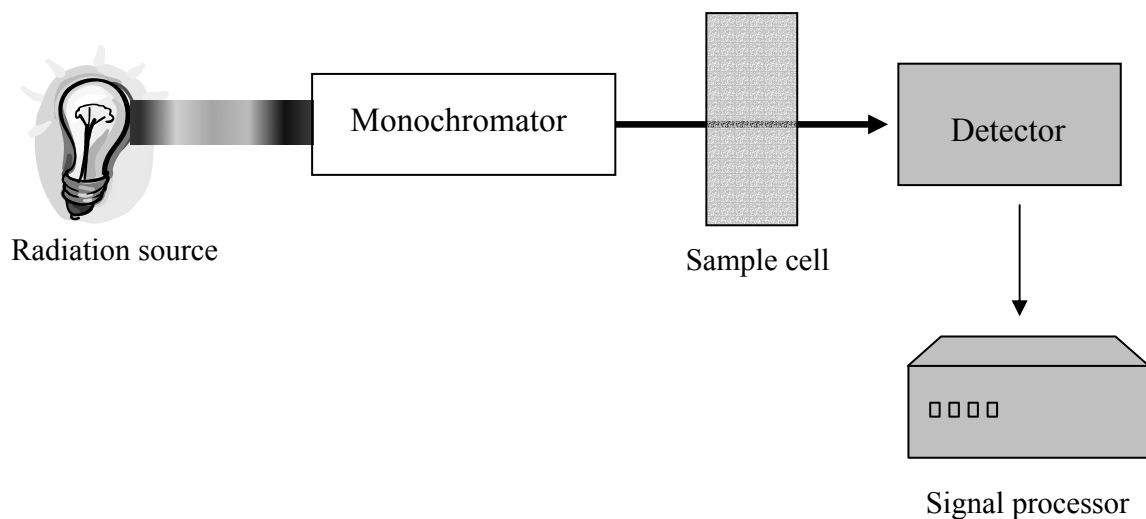
(2) Phosphorescence - emission of photon during transition between states with different spin quantum #'s (e.g. $T_1 \rightarrow S_0$)



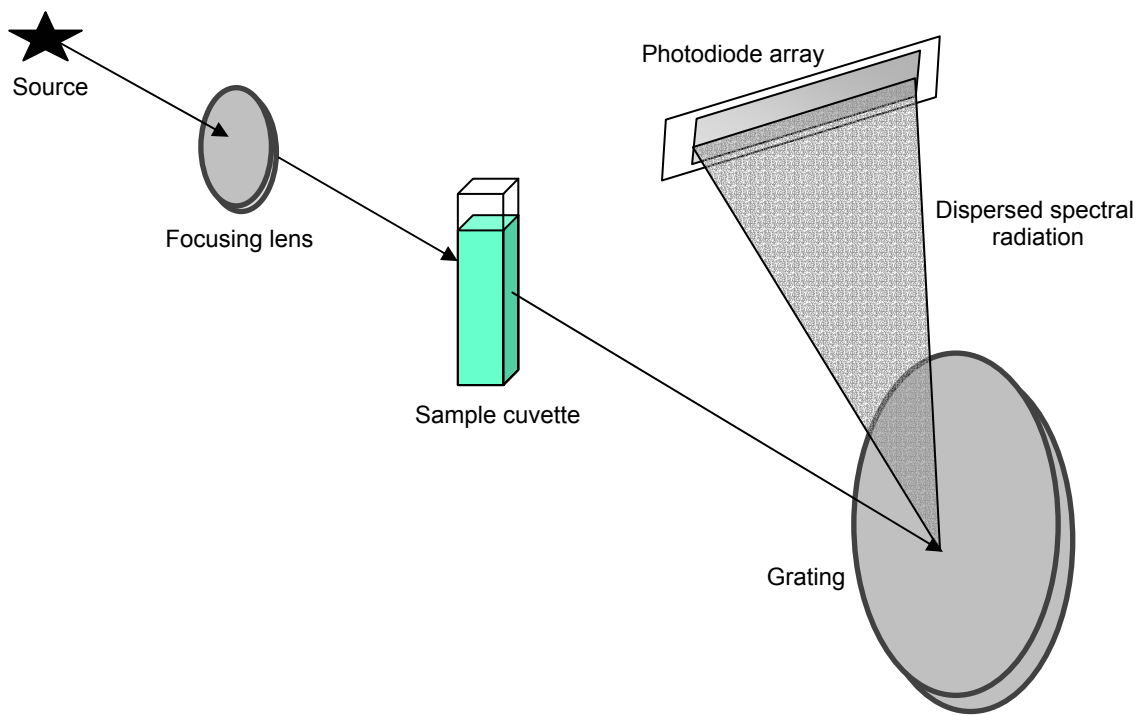
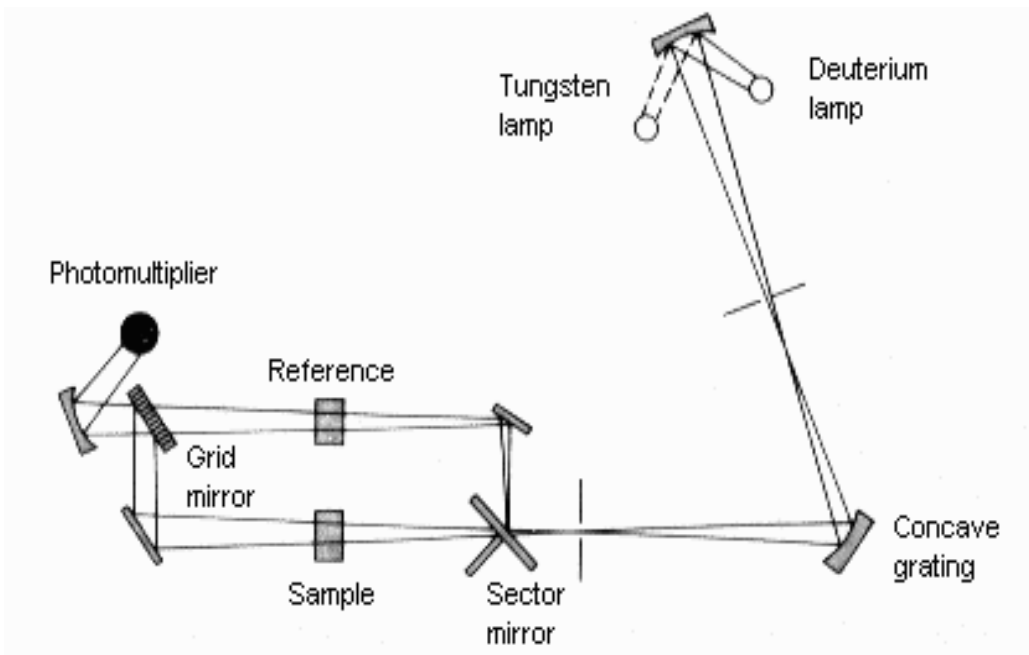
6. Instrumentation

(a) Basic components

- (1) Radiation source – Visible = tungsten filament lamp; UV = deuterium and hydrogen lamps
- (2) Monochromators/filters – Restricts wavelength to offer narrow band of radiation
- (3) Sample containers (cuvettes or cells) – glass, plastic or quartz (1 cm)
- (4) Phototubes/photomultiplier tubes – emit photoelectrons after being irradiated, current is then amplified and measured



- Single Beam Instruments
- Double Beam Instruments
- Array Detector Instruments



7. Scope - Application to absorbing species

<u>Chromophore</u>	<u>Example</u>	<u>Solvent</u>	<u>λ_{\max}</u>
Alkenes	$C_6H_{13}CH=CH_2$	n-Heptane	177 nm
Alkynes	$CH_2=CHCH=CH_2$	n-Heptane	196 nm
Nitro	CH_3NO_2	Isooctane	280 nm
Nitrate	$C_2H_5ONO_2$	Dioxane	270 nm

Absorption by inorganic ions (η to σ^*)

Carbonate, 217 nm; nitrite, 313 nm; azido, 230 nm

Absorption due to σ to σ^*

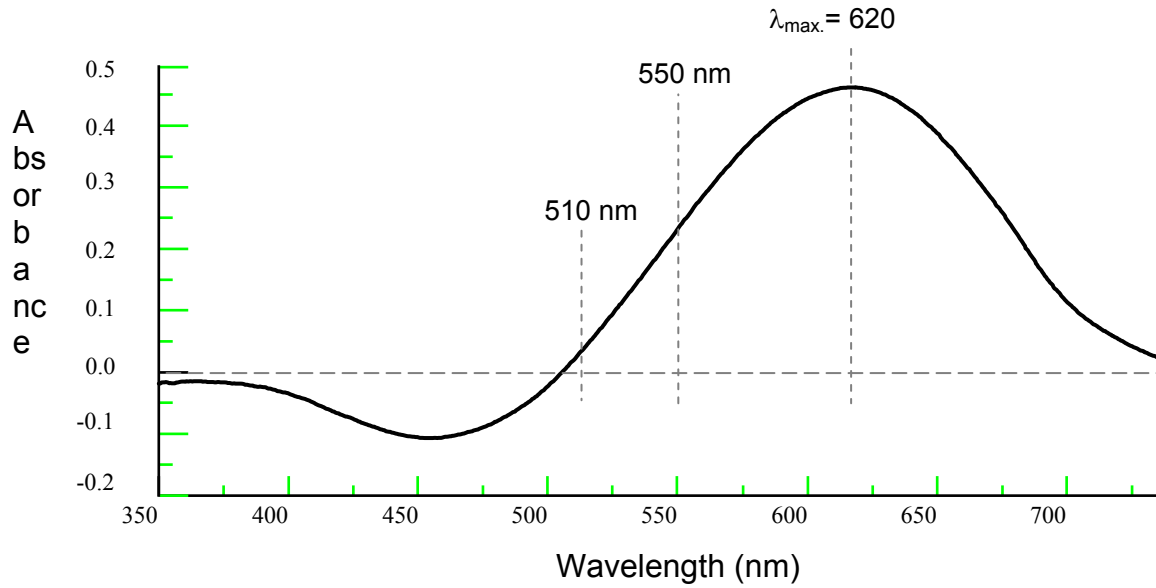
Methane, 125 nm; ethane, 135 nm

Absorption of aromatic compounds

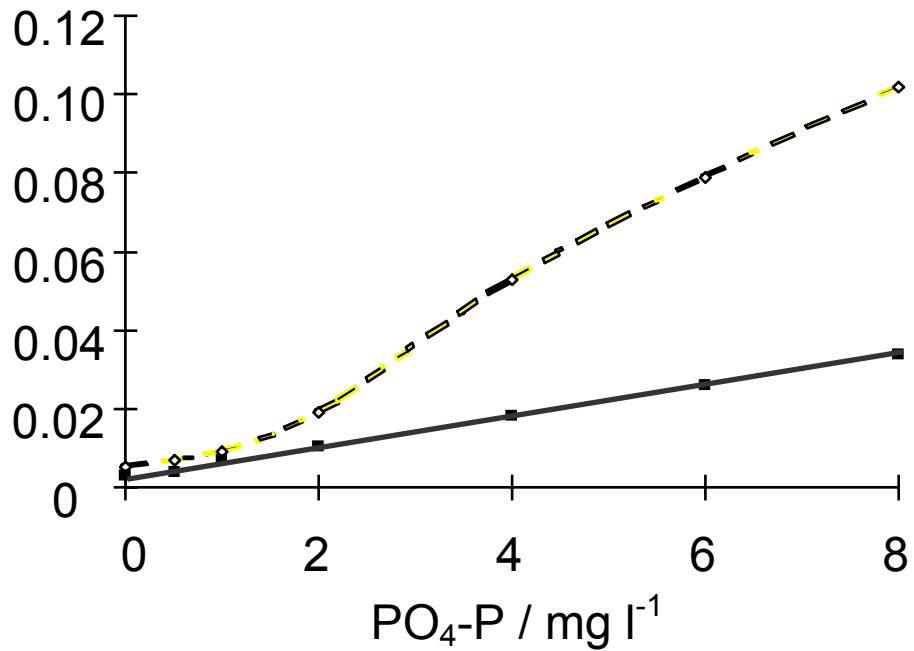
<u>Compound</u>	<u>Formula</u>	<u>λ_{\max}</u>
Benzene	C_6H_6	256
Toluene	$C_6H_5CH_3$	261
Phenol	C_6H_5OH	270
Styrene	$C_6H_5CH=CH_2$	282

8. Optimizing Experimental Conditions

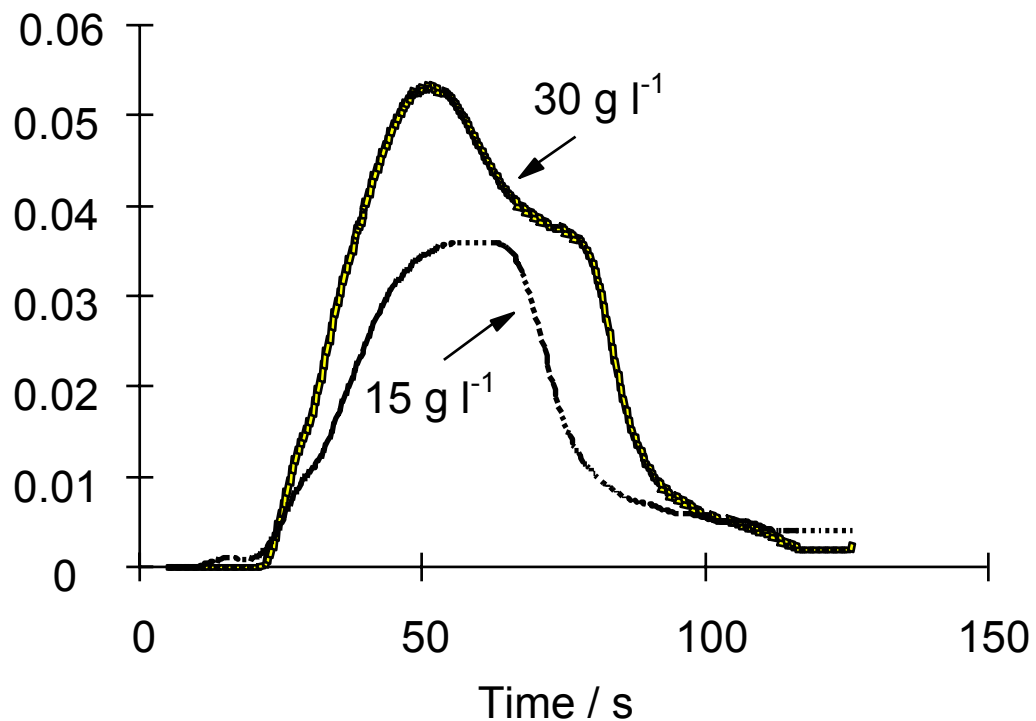
a. Wavelength Selection



b. pH of Solution



c. Reagent Concentration



d. Temperature

e. Interfering Substances

9. Applications

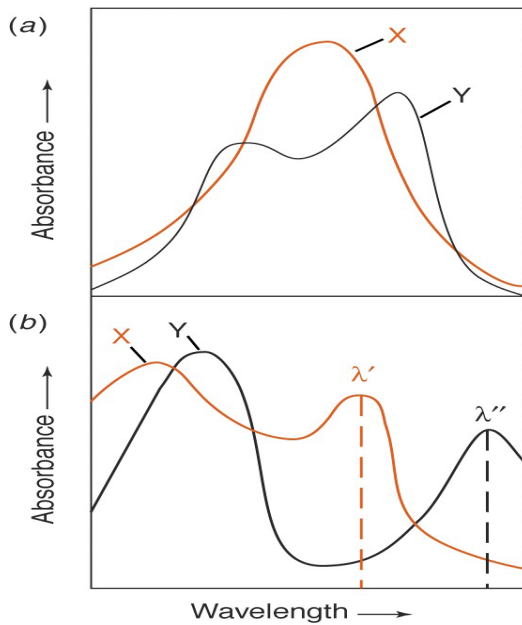
a. Health Sciences – 95% of all analyses are performed by spectrophotometry.

b. Biological Sciences

c. Chemical & Environmental Sciences

Organic, inorganic systems and biochemical systems

10. Analysis of Mixtures

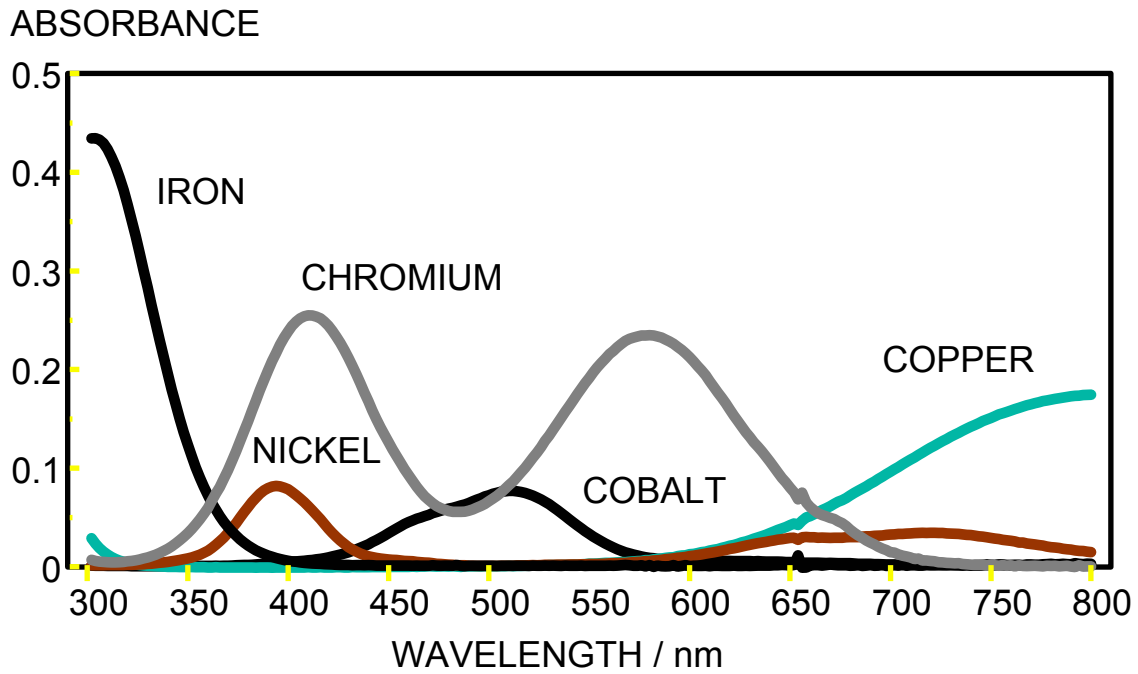


$$\text{Mixture}_{\text{Abs}} = \epsilon_x b[x] + \epsilon_Y b[Y] + \dots$$

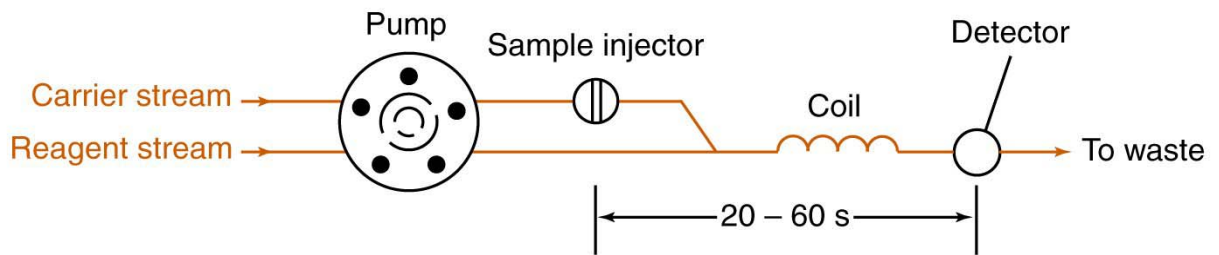
where ϵ = molar absorptivity of each species at wavelength specified
 b = cell pathlength

-measure A at more wavelengths than there are components in mixture

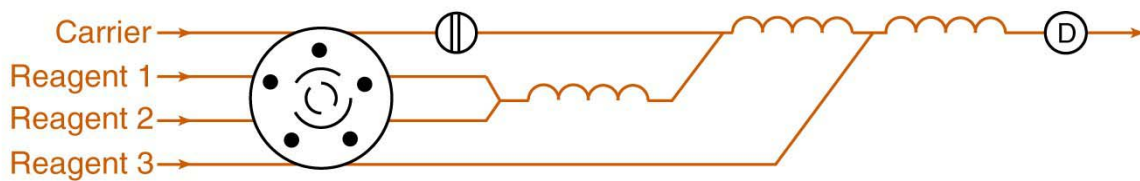
Ex. Two-component mixture, at least three wavelengths. More is better – increase in accuracy.



11. Flow Injection Analysis



(a)



(b)

