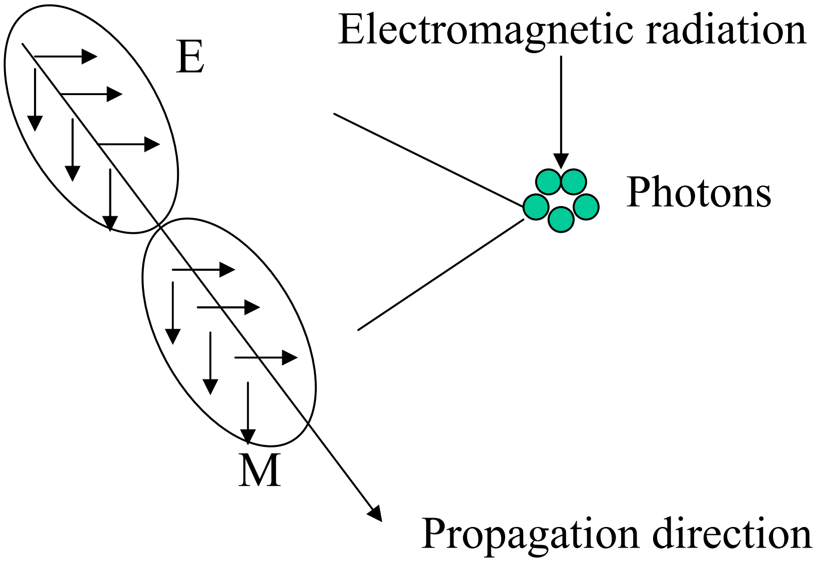
Spectrophotometry – Fundamentals

1. Electromagnetic radiation – properties



Relationships

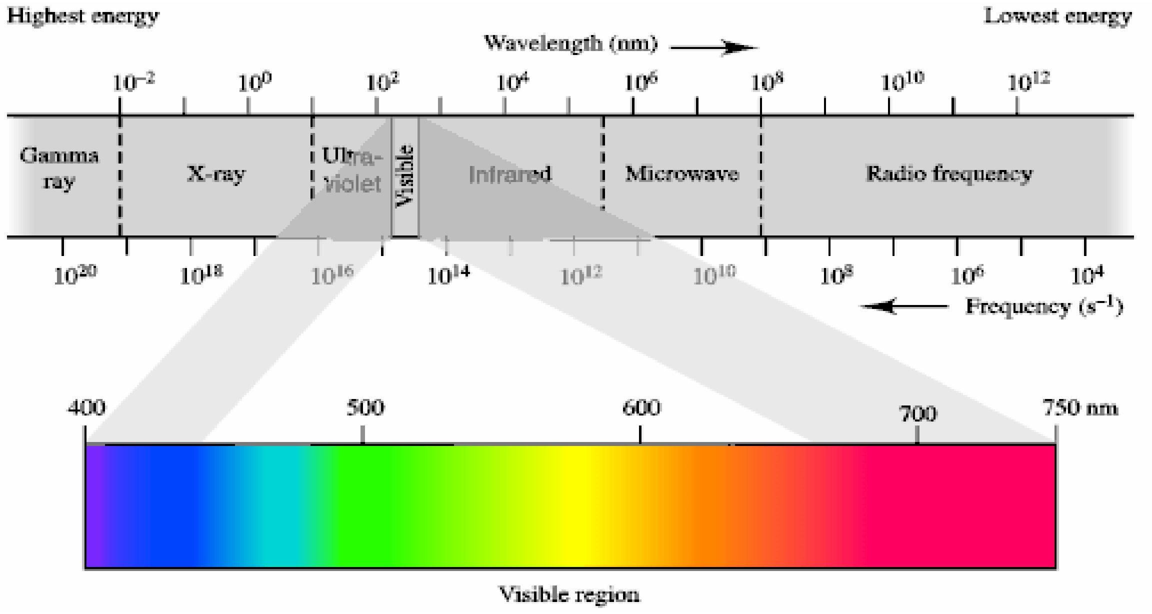
a. Frequency/Wavelength - $\nu\lambda = c$
where c = the speed of light in vacuum (2.998×10^{8} m/s)

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

2. Electromagnetic Spectrum



Why is a red solution red?

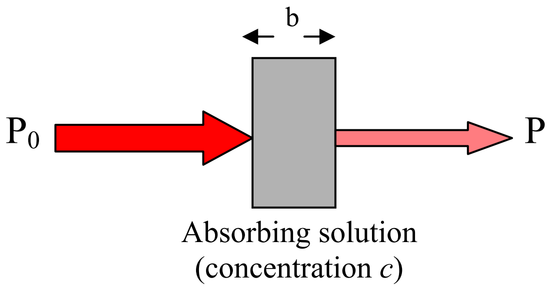
e.g. $FeSCN^{2+}$

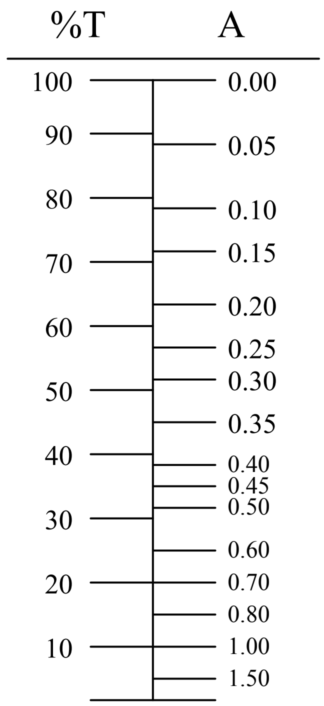
Wavelength (nm)	Color	Complementary color
400-435	Violet	Yellow-green
435-480	Blue	Yellow
480-490	Green-blue	Orange
490-500	Blue-green	Red
500-560	Green	Purple

3. Absorption of Light

Term & Symbol*	Definition	Alt. Name & Symbol
Radiant power P, P_0	Energy of radiation	Radiation intensity I, I_0
Absorbance A	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





Transmittance $T = P/P_0$

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

Beer's Law

a. Beer's Law

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

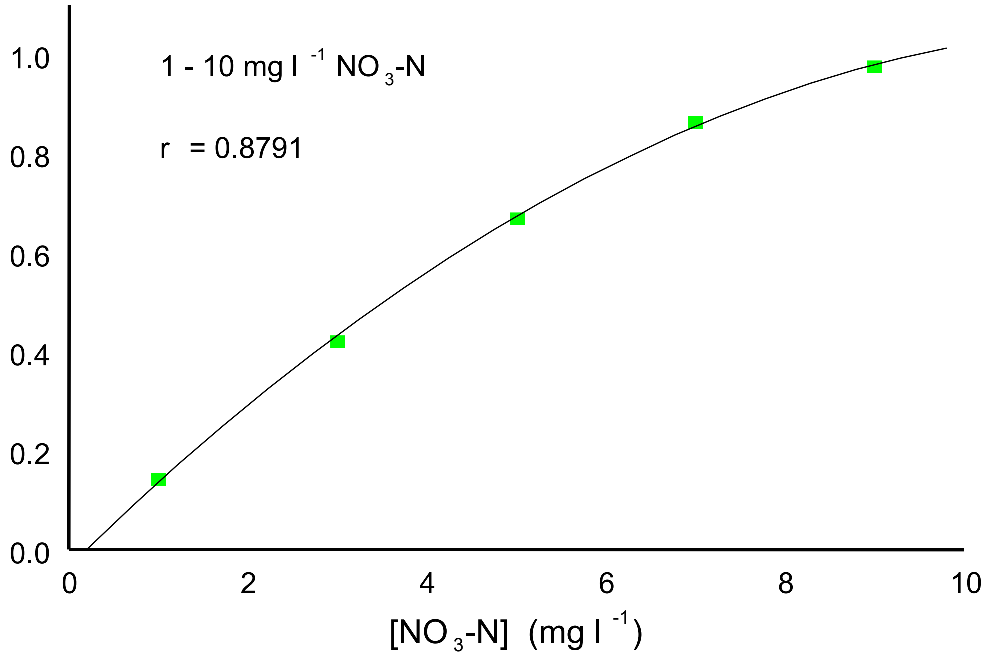
Ex: A 7.50×10^{-5} M solution of $KMnO_4$ has T of 36.4% when measured in a 1.05-cm cell at 525nm.

(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.

$$HNO_2 + H_2O \longleftrightarrow H_3O + NO_2^-$$

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

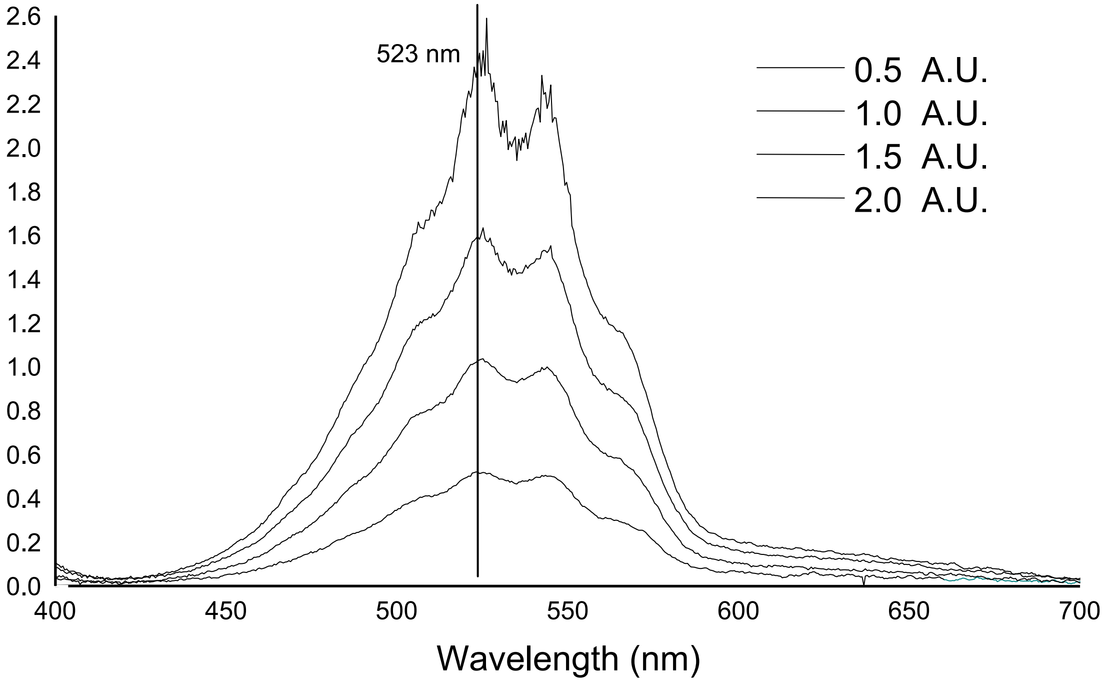
- Use of filters, diffraction gratings
- Molar absorptivities must be equal

Stray radiation

$$A = \log \frac{P_o}{P} \qquad\qquad A = \log \frac{P_o + P_s}{P + P_s}$$

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:

$$\mathrm{M} + h\nu \rightarrow \mathrm{M}^* \quad \text{(excited state)}$$

$$\mathrm{M}^* \rightarrow \mathrm{M} + \text{heat} \quad \text{(relaxation)}$$

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

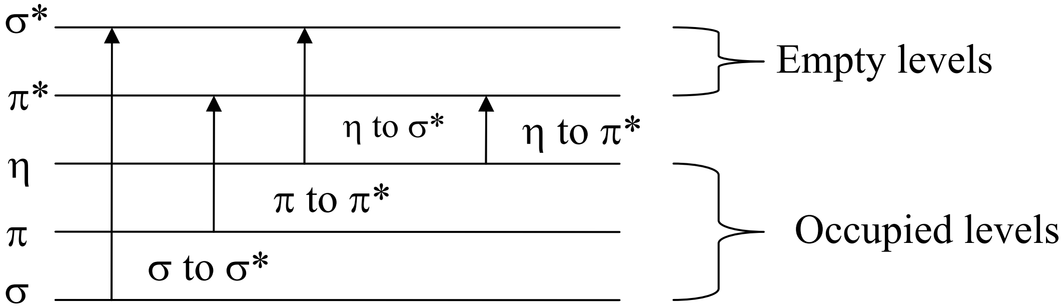
σ to σ^* ⟶ Alkanes

σ to π^* ⟶ Carbonyl compounds

π to π^* ⟶ Alkenes, carbonyl compounds, alkynes, azo compounds

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

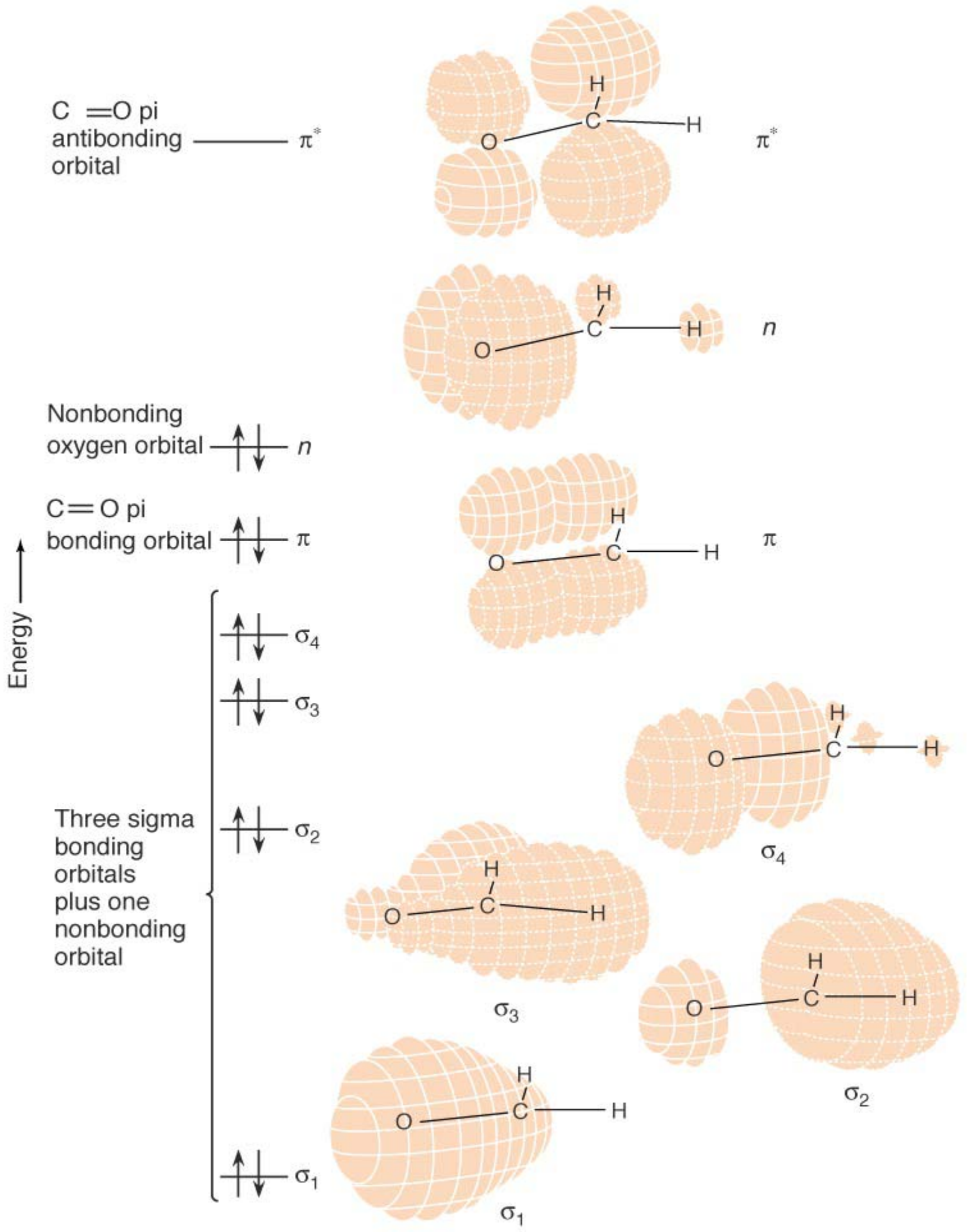
η to π^* ⟶ Carbonyl compounds



Highest occupied molecular orbital (HOMO)

Lowest unoccupied molecular orbital (LUMO)

$$E = h\nu = h v/\lambda = E\,(\mathrm{LUMO}) - E\,(\mathrm{HOMO})$$

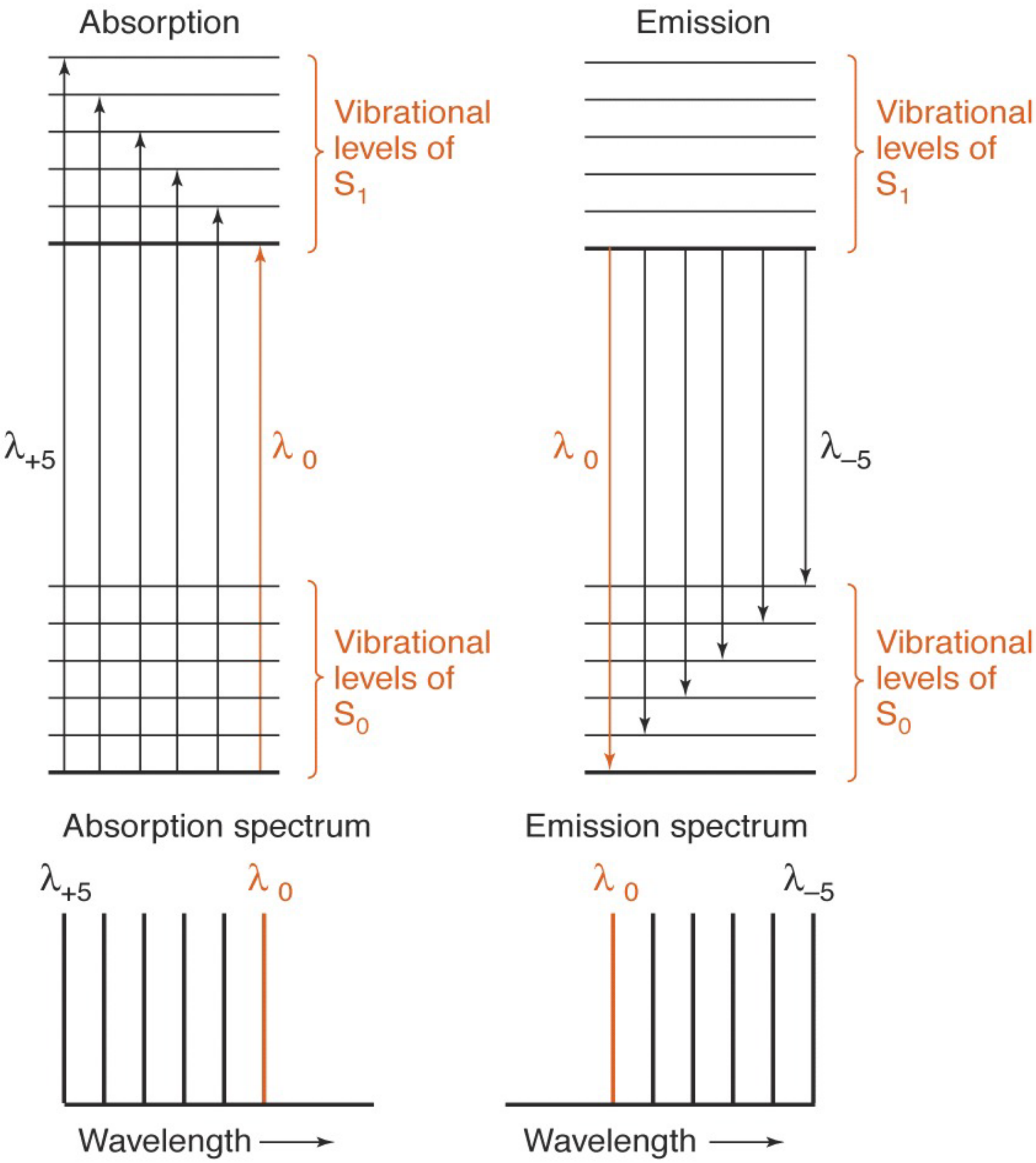


Therefore - Overall change in Energy with an orbital of a molecule is:

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

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

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


Absorption
Emission
Vibrational levels of S_1
Vibrational levels of S_1
λ_{+5}
λ_0
λ_0
λ_{-5}
Vibrational levels of S_0
Vibrational levels of S_0
Absorption spectrum
Emission spectrum
λ_{+5}
λ_0
λ_0
λ_{-5}
Wavelength →
Wavelength →

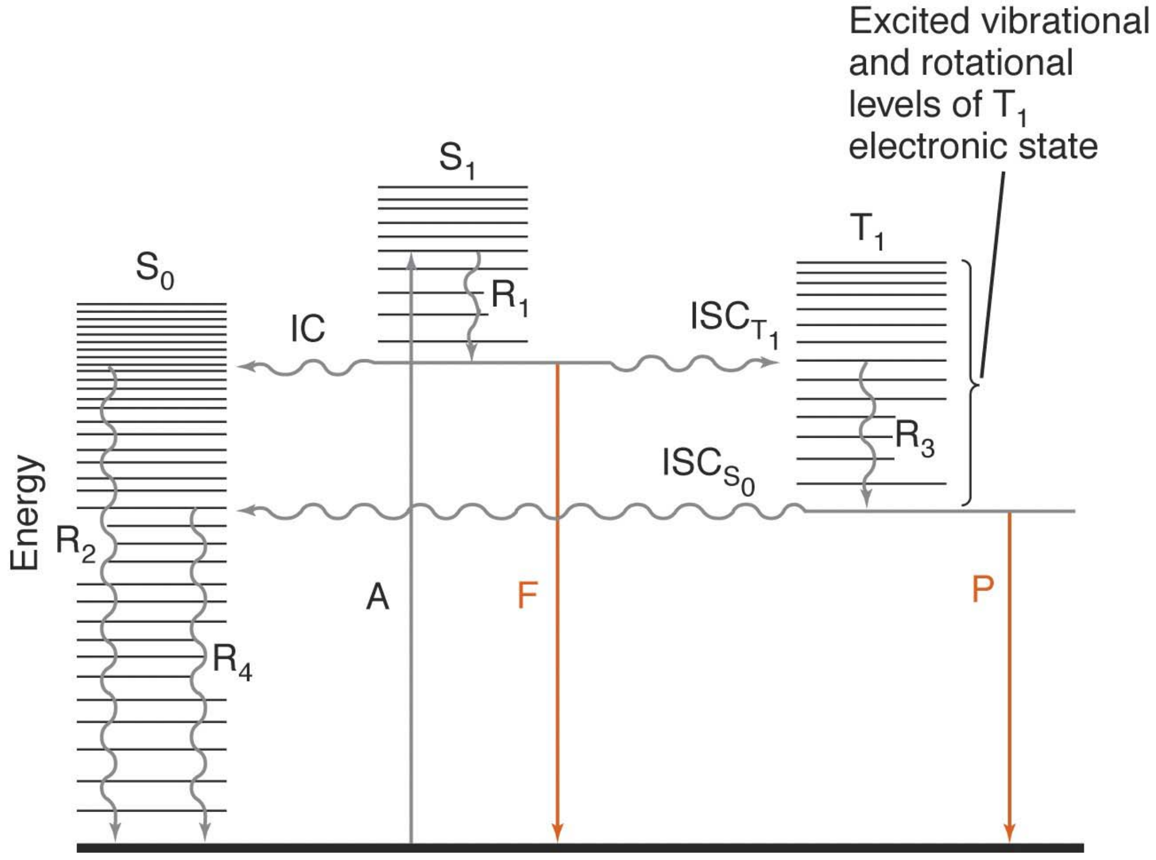
Emission Processes

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. $S_1 \rightarrow S_0$)

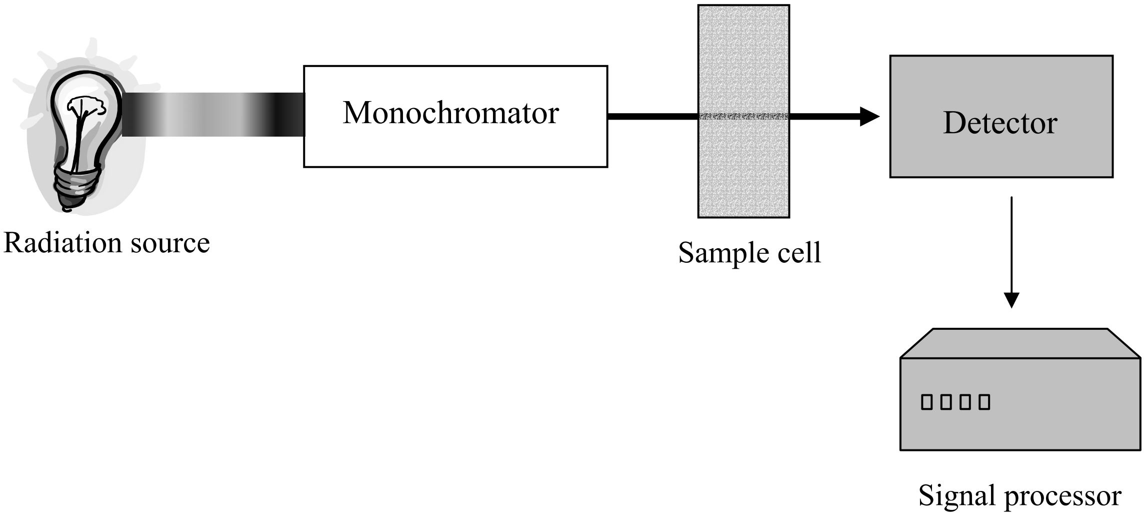
(2) Phosphorescence - emission of photon during transition between states with different spin quantum #'s (e.g. $T_1 \rightarrow S_0$)



6. Instrumentation

(a) Basic components

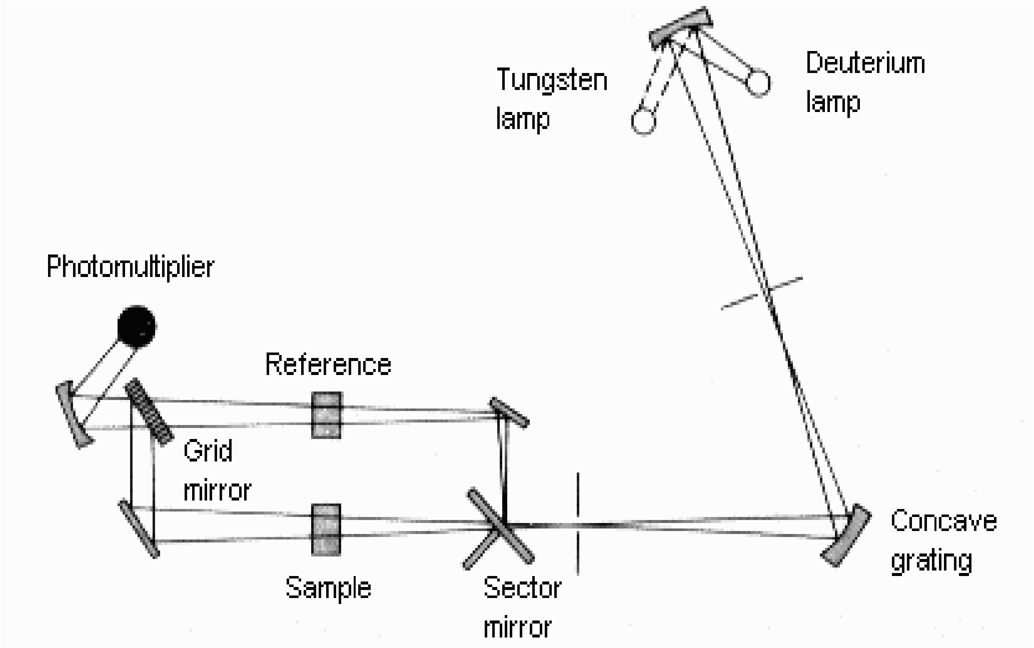
(1) Radiation source – Visible = tungsten filament lamp; UV = deuterium and hydrogen lamps
(2) Monochromators/filters – Restricts wavelength to offer narrow band of radiation
(3) Sample containers (cuvettes or cells) – glass, plastic or quartz (1 cm)
(4) Phototubes/photomultiplier tubes – emit photoelectrons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

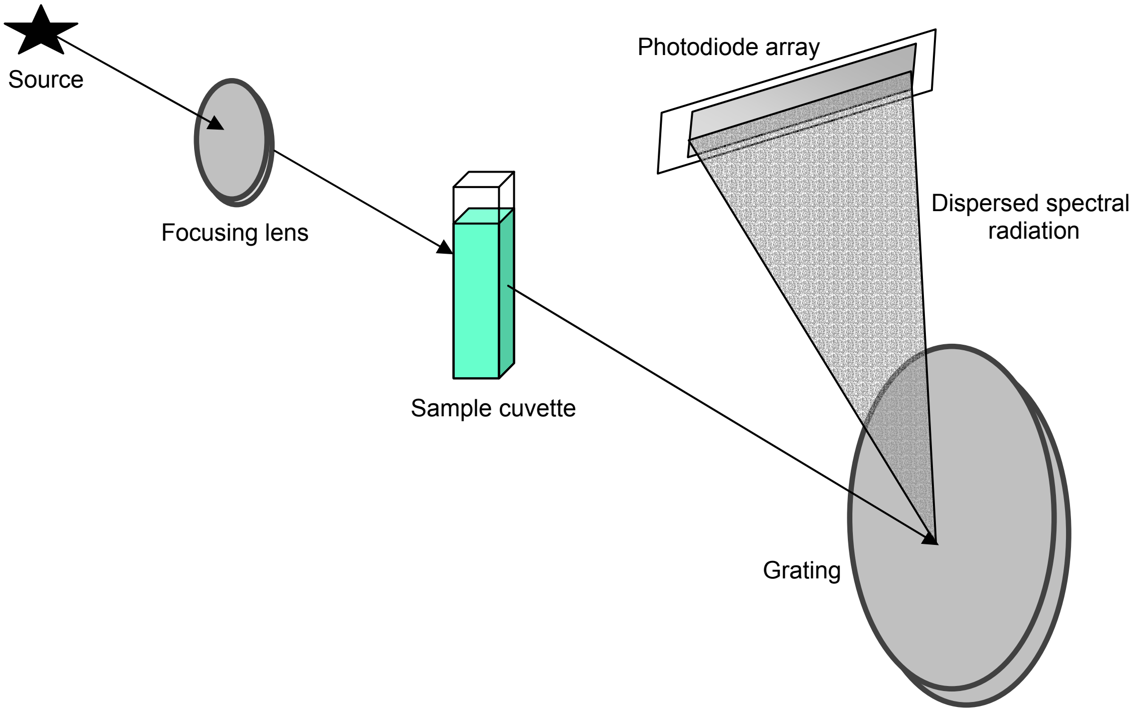


- Single Beam Instruments

- Double Beam Instruments

- Array Detector Instruments


Tungsten lamp
Deuterium lamp
Photomultiplier
Reference
Grid mirror
Concave grating
Sample
Sector mirror


Source
Photodiode array
Focusing lens
Dispersed spectral radiation
Sample cuvette
Grating

7. Scope - Application to absorbing species

Chromophore	Example	Solvent	λmax
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

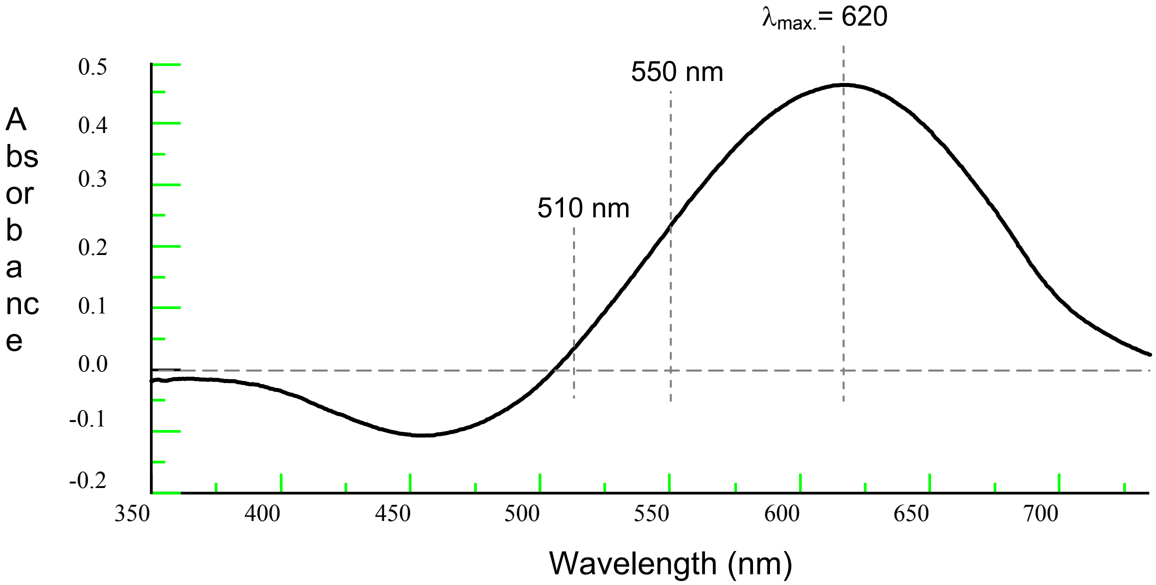
Absorption due to σ to σ*
Methane, 125 nm; ethane, 135 nm

Absorption of aromatic compounds

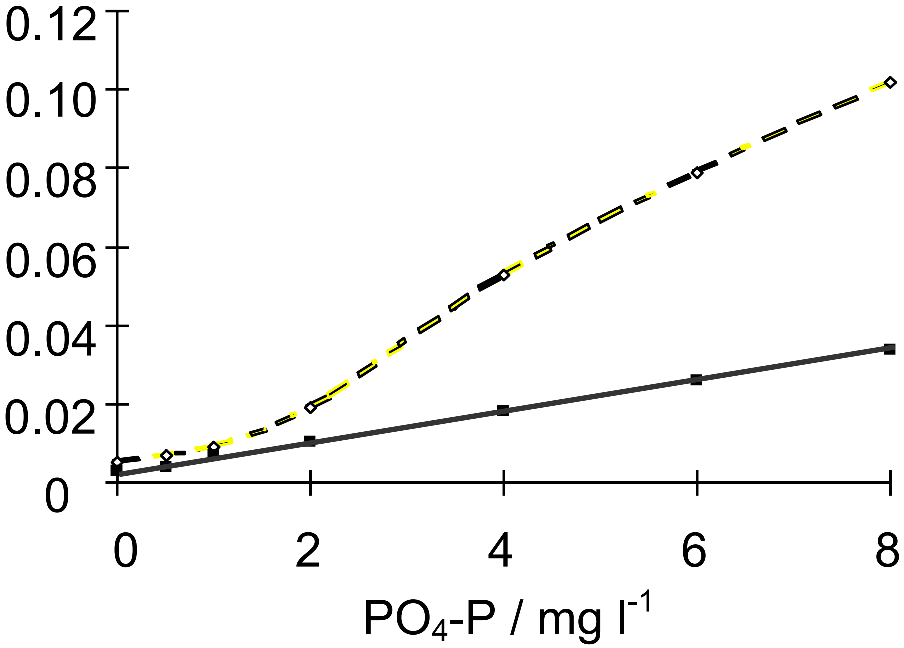
Compound	Formula	λmax
Benzene	C_6H_6	256
Toluene	$C_6H_5CH_3$	261
Phenol	C_6H_5OH	270
Styrene	$C_6H_5CH=CH_2$	282

8. Optimizing Experimental Conditions

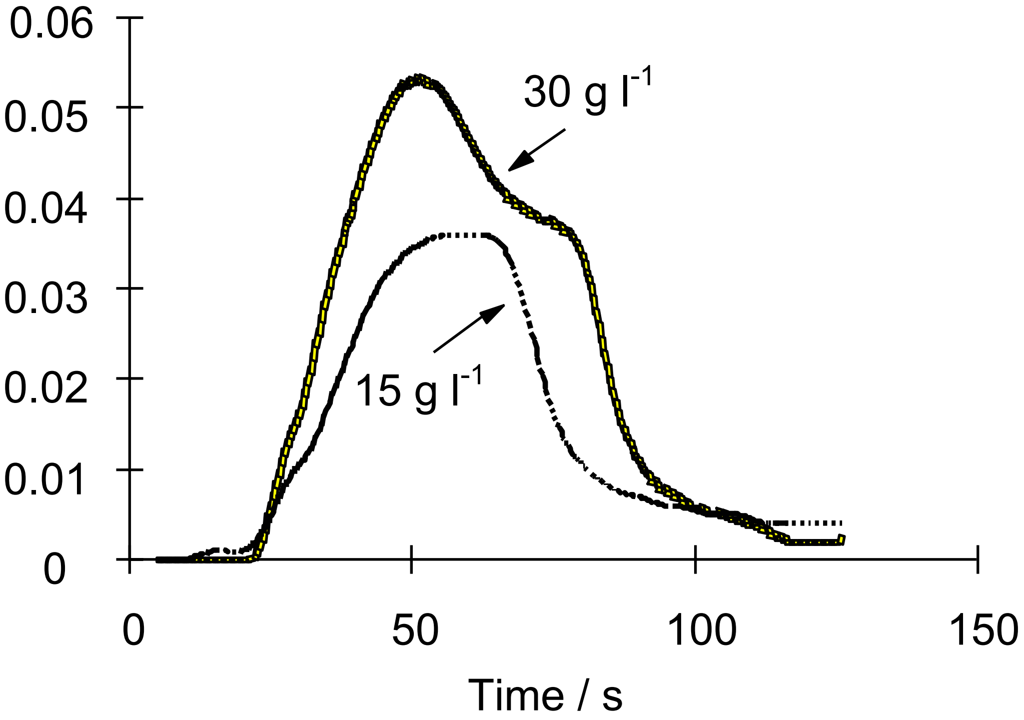
a. Wavelength Selection



b. pH of Solution



c. Reagent Concentration



d. Temperature
e. Interfering Substances

9. Applications

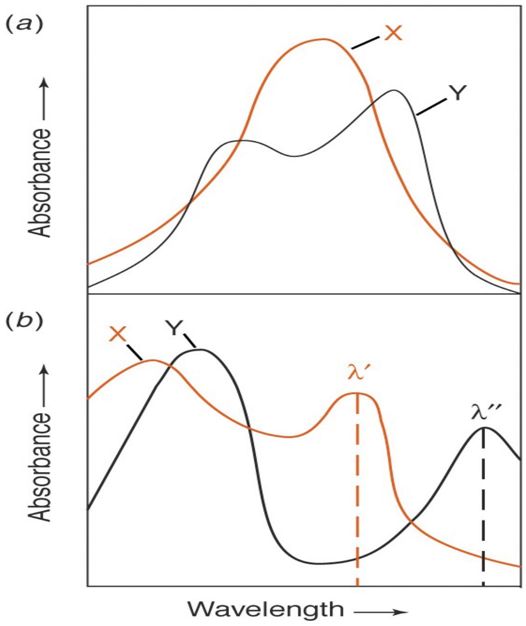
a. Health Sciences – 95% of all analyses are performed by spectrophotometry.

b. Biological Sciences

c. Chemical & Environmental Sciences

Organic, inorganic systems and biochemical systems

10. Analysis of Mixtures

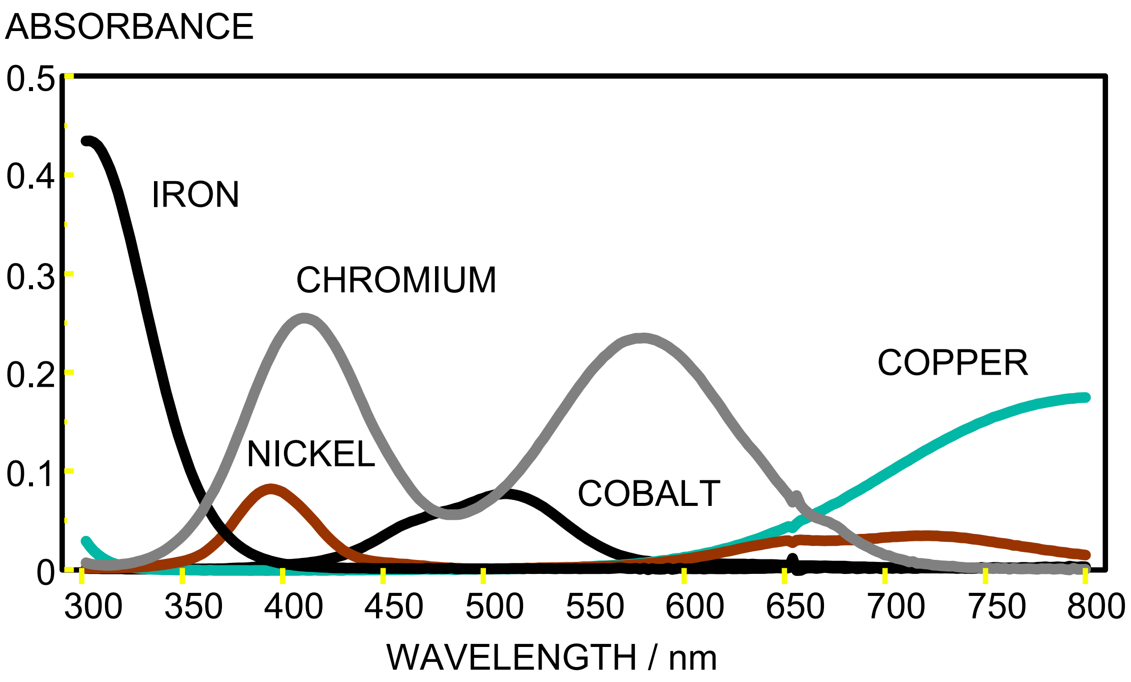


$$\text{Mixture}_{\text{Abs}} = \varepsilon_X b[x] + \varepsilon_Y b[Y] + \ldots\ldots$$

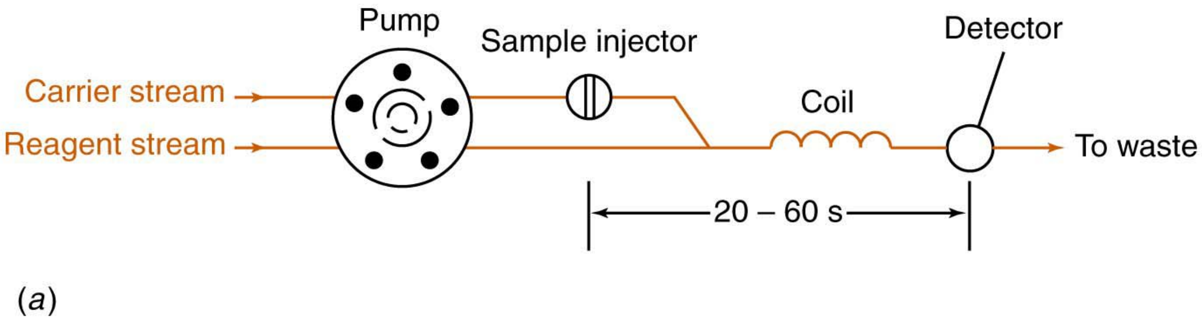
where ε = molar absorptivity of each species at wavelength specified
b = cell pathlength

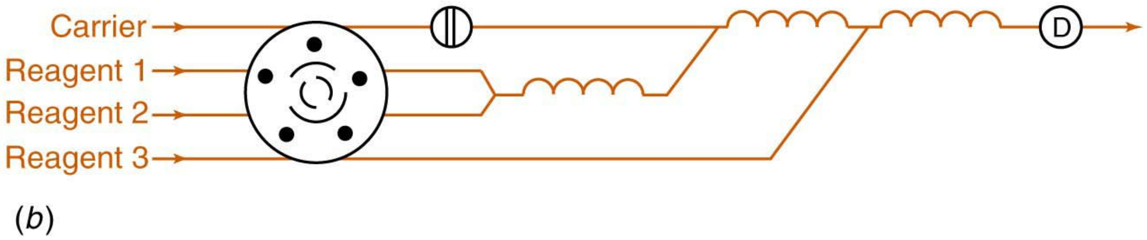
-measure A at more wavelengths then there are components in mixture

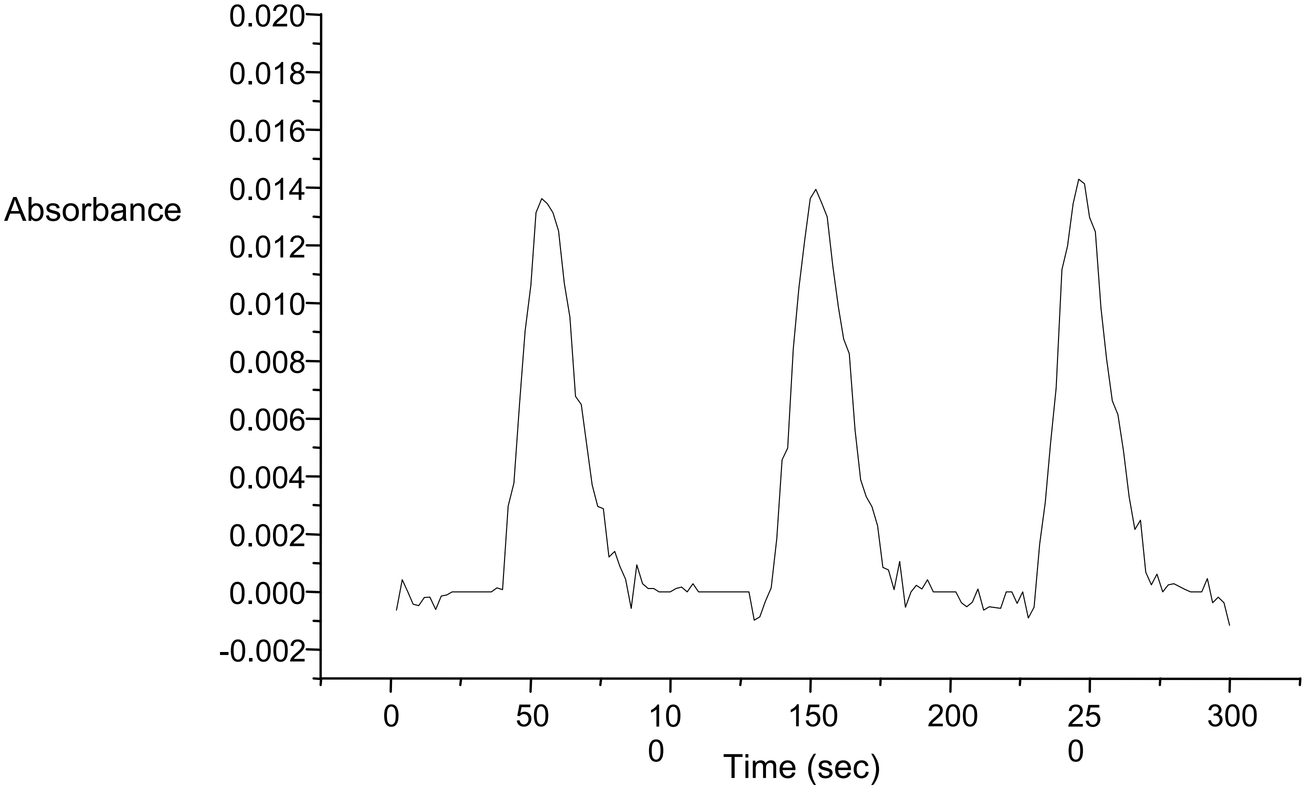
Ex. Two-component mixture, at least three wavelengths. More is better – increase in accuracy.



11. Flow Injection Analysis







Absorbance
0.020
0.018
0.016
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
-0.002
0
50
10
0
150
200
25
0
300
Time (sec)