CHE680
Advanced Analytical Chemistry
Lecture 5

Buffalo State
State University of New York

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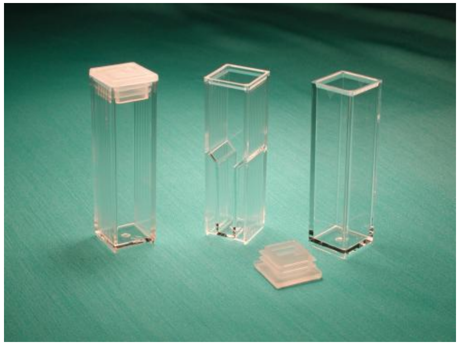
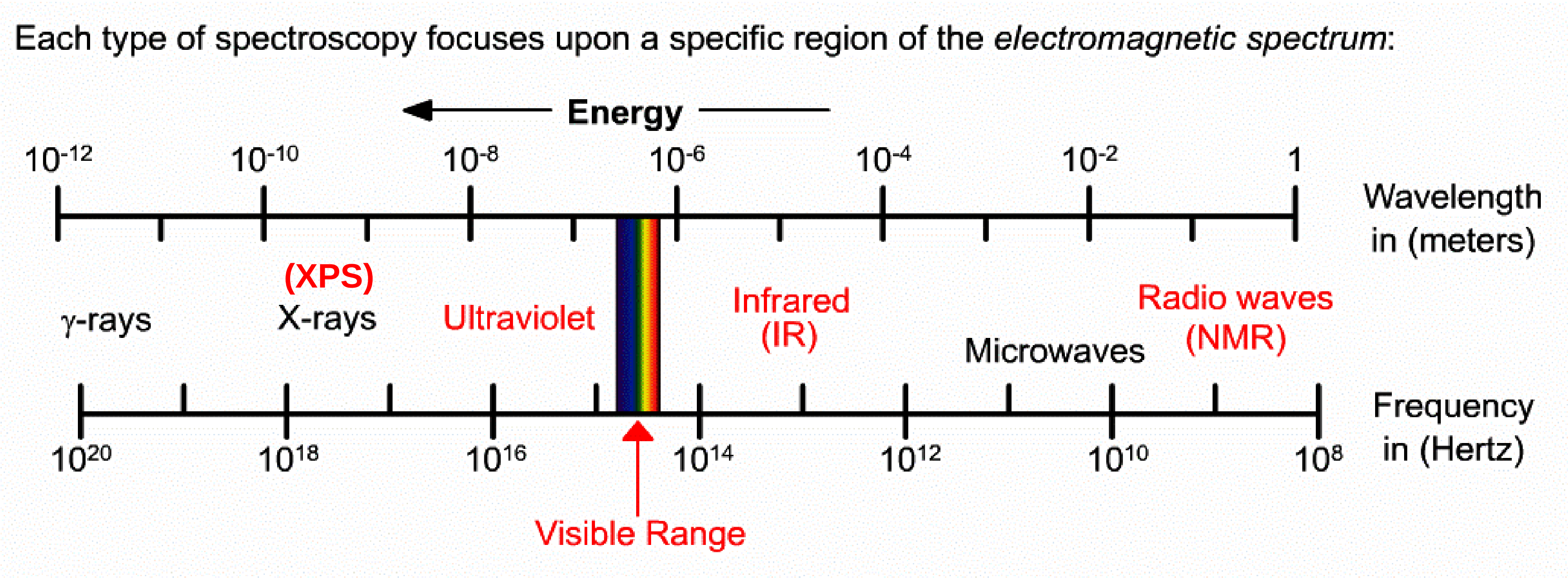
UV/Vis Spectroscopy

- Theory
- Chemical Information
- Instrumentation



http://www.cem.msu.edu/~reusch/VirtTxtJml/Spectrpy/UV-Vis/spectrum.htm
http://www.shu.ac.uk/schools/sci/chem/tutorials/molspec/uvvisab1.htm

UV-Vis



Each type of spectroscopy focuses upon a specific region of the *electromagnetic spectrum*:

$c = \nu \cdot \lambda$, $\nu = c/\lambda$, or $\lambda = c/\nu$

$$\bar{\nu} = \frac{1}{\lambda(m)} \cdot \frac{1m}{100cm}$$

XPS (*eV*)
UV/Vis (nm)
IR (cm^{-1})
NMR (MHz)

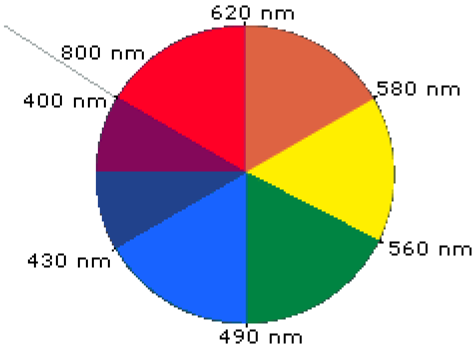
1.0 *eV* = 1240 nm = 1.24 μm = 8065.5 cm^{-1} = 2.43×10^{9} MHz

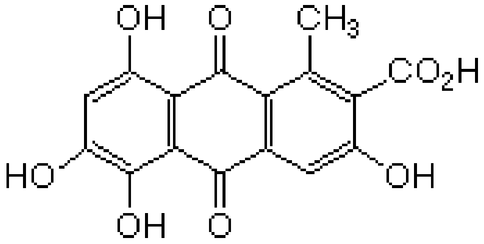
Why should we learn this stuff? After all, nobody solves structures with UV any longer!

- Many organic molecules have chromophores that absorb UV
- UV absorbance is about 1000 x easier to detect per mole than NMR
- Still used in following reactions where the chromophore changes. Useful because timescale is so fast, and sensitivity so high. Kinetics, esp. in biochemistry and enzymology.
- Most quantitative analytical chemistry in organic chemistry is conducted using HPLC with UV detectors
- Affects quantitative interpretation of HPLC peak heights

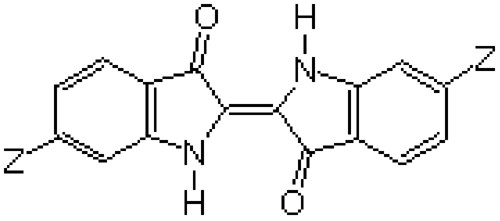
Compounds with/without Colors

Some Natural Organic Pigments



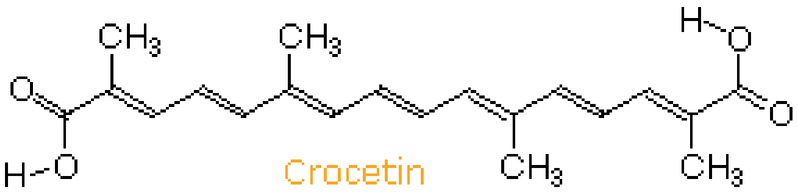


Kermesic Acid
(Carminic Acid)
from the insect *Coccus cacti*

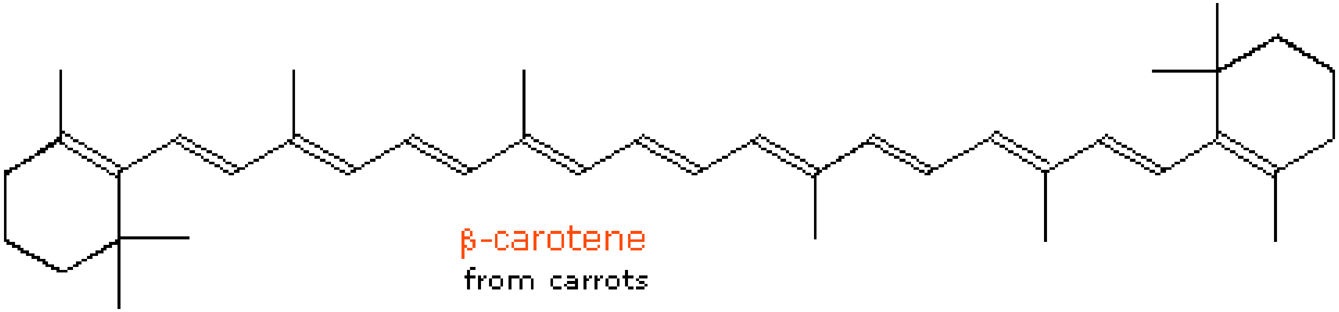


Z=H
Indigo
from *Isatis tinctoria* (woad)

Z=Br
Punicin or Tyrian Purple
from mollusks of the genus *Murex*



Crocetin
from saffron



β-carotene
from carrots

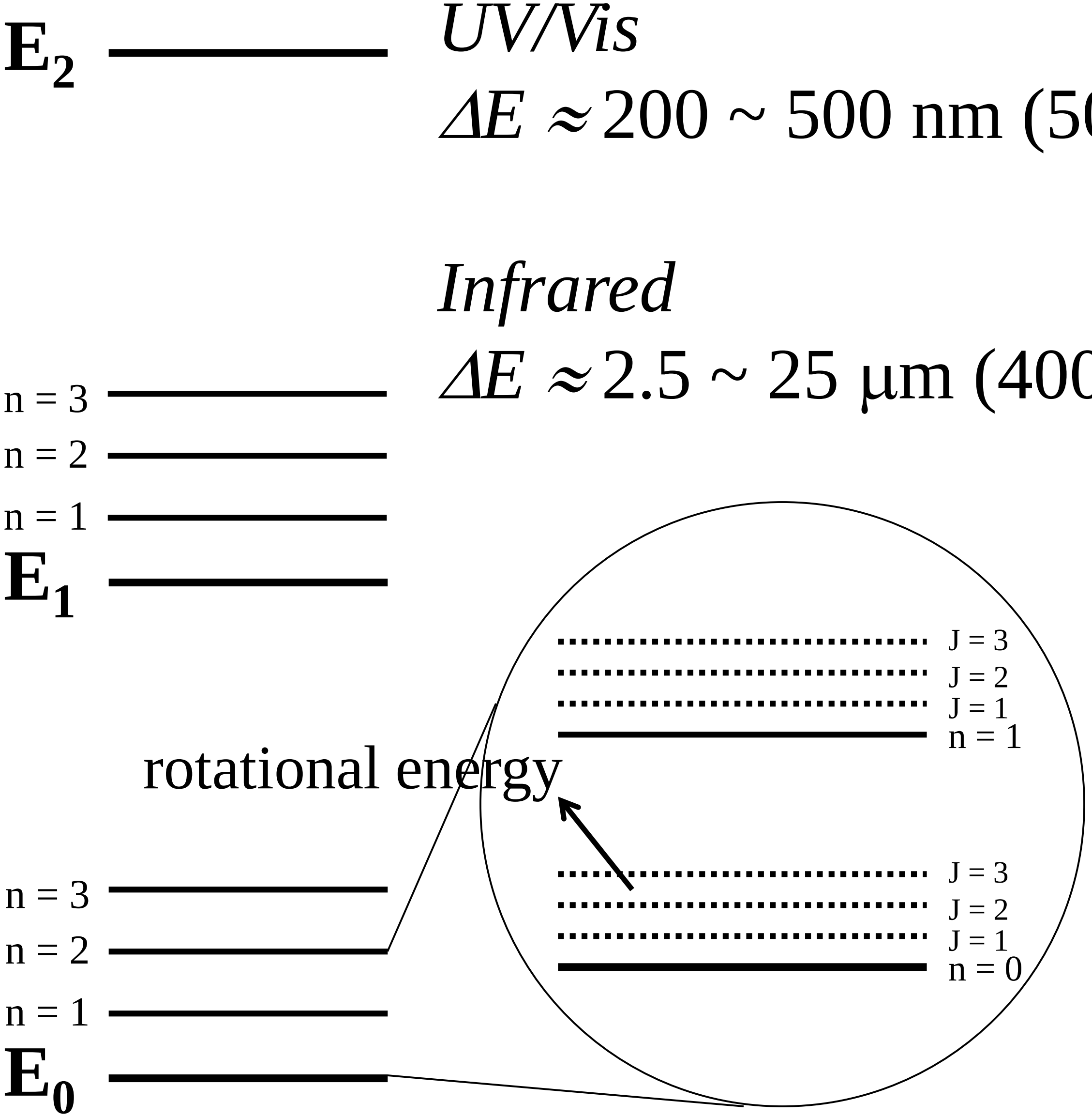
Relative Energy Level

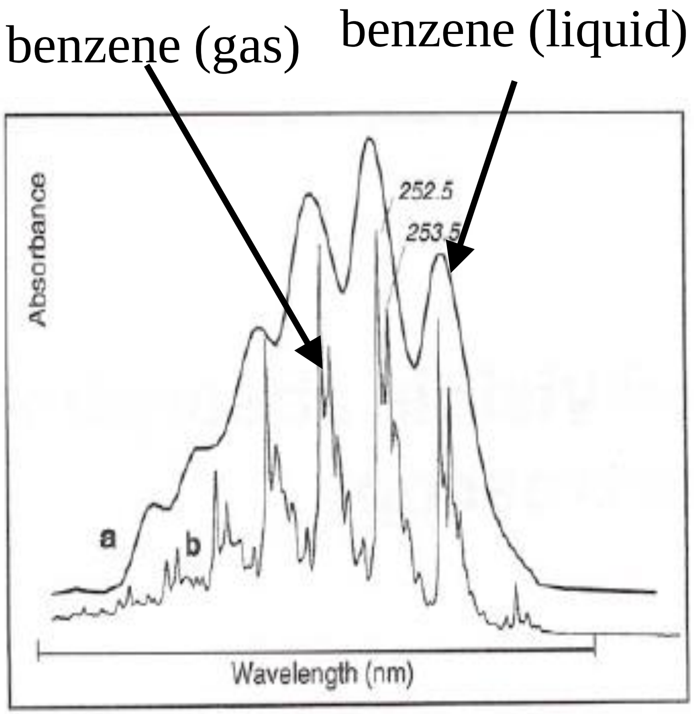
UV/Vis

$\Delta E \approx$ 200 ~ 500 nm (50000 ~ 20000 cm^{-1})

Infrared

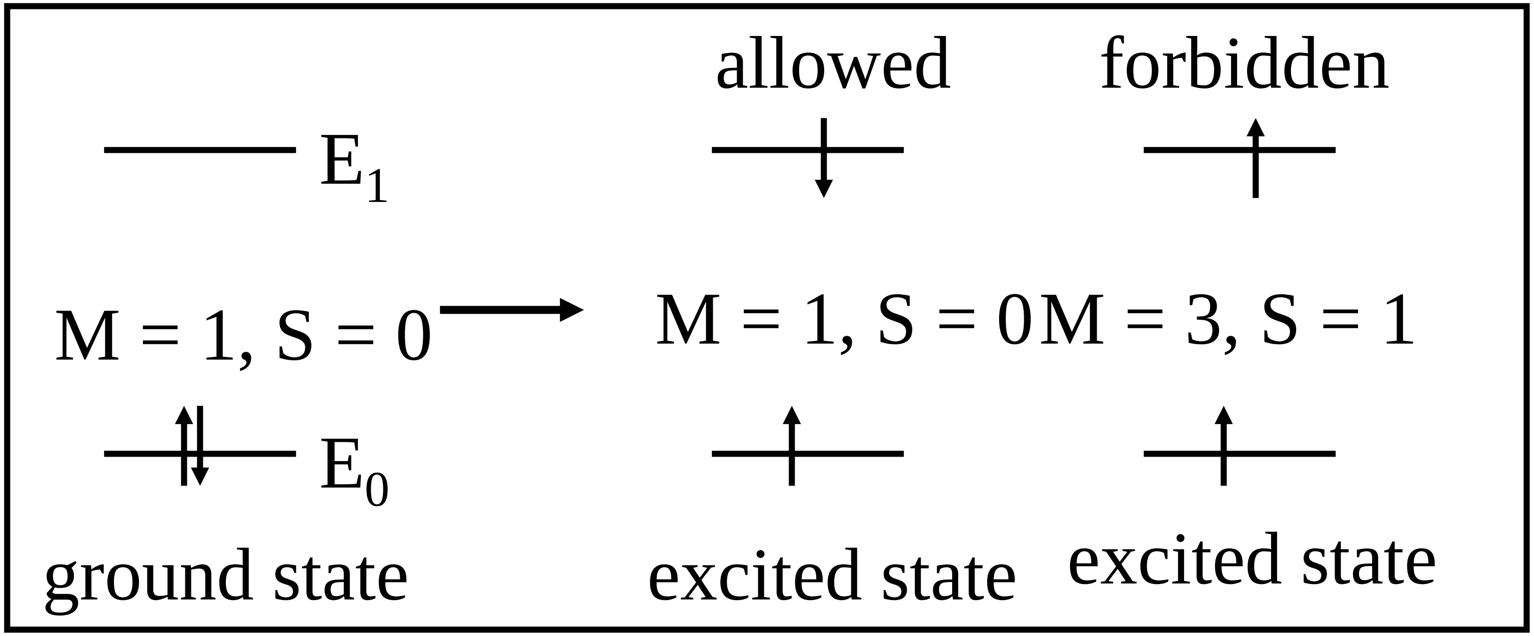
$\Delta E \approx$ 2.5 ~ 25 µm (4000 ~ 400 cm^{-1})





Spin Selection Rule: $\Delta S = 0$

- M (multiplicity) = 2S+1 (S = total spin)
- Allow S→S, and T→T processes but not S→T and T→S.
- Ground states are usually singlets; thus most excitations are to singlet excited states, like $S_0 \rightarrow S_1$, $S_0 \rightarrow S_2$, …



allowed

forbidden

E_1

M = 1, S = 0

M = 1, S = 0

M = 3, S = 1

E_0

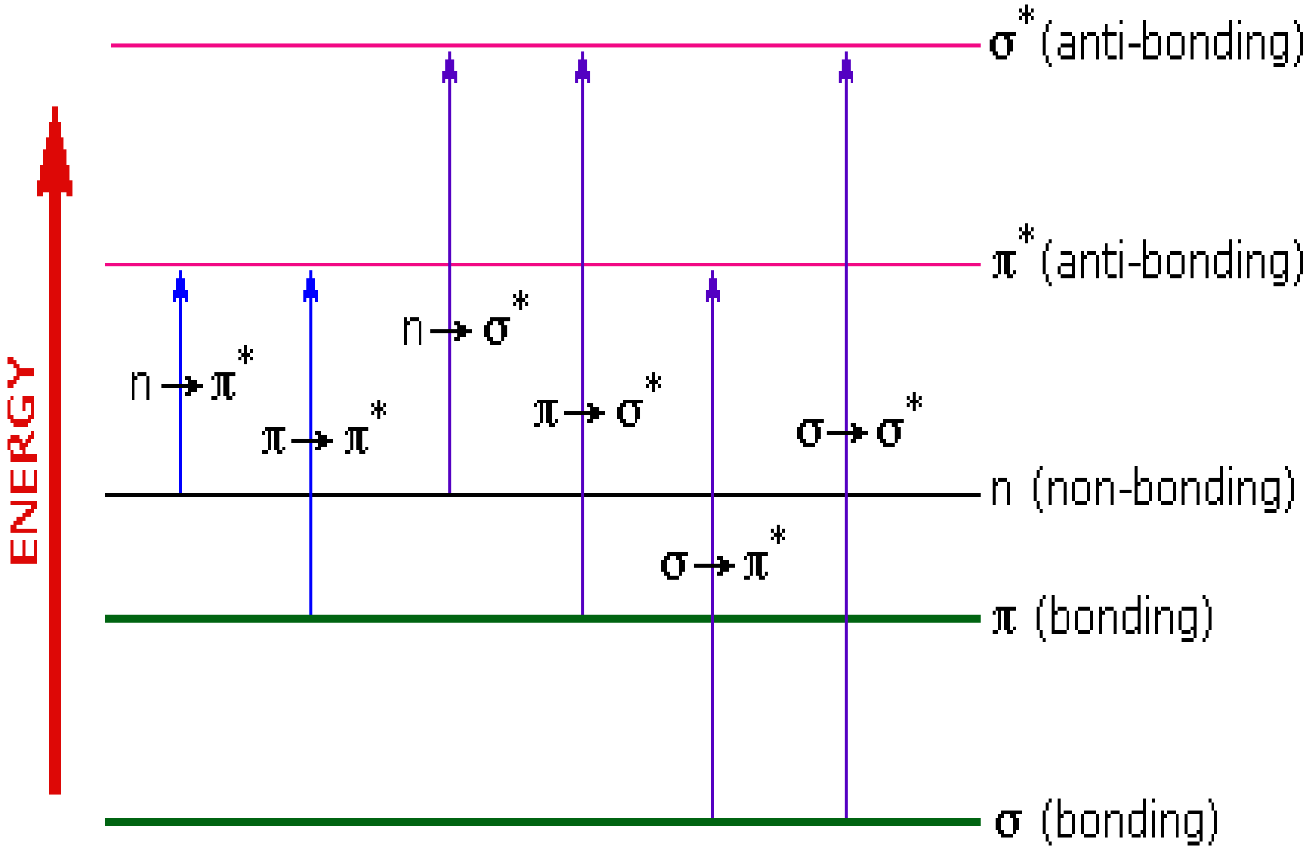
ground state

excited state

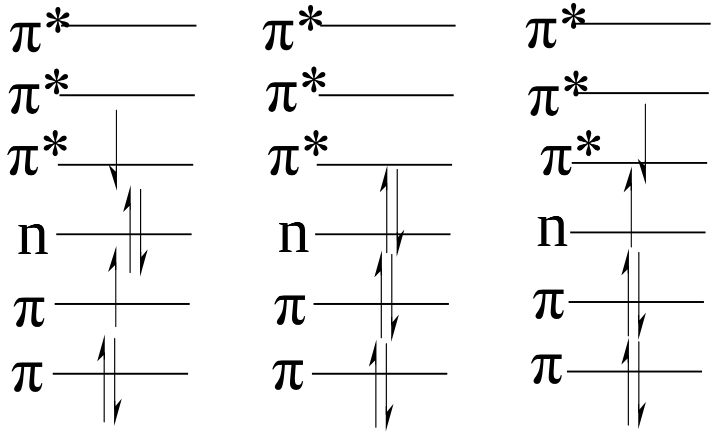
excited state

Possible Electronic Transitions 1

UV/vis spectroscopy probes the electronic transitions of molecules involving π, σ, and *n* electrons as they absorb light in the UV and visible regions of the electromagnetic spectrum.



What are the nature of these absorptions?



Example
for a
simple
enone

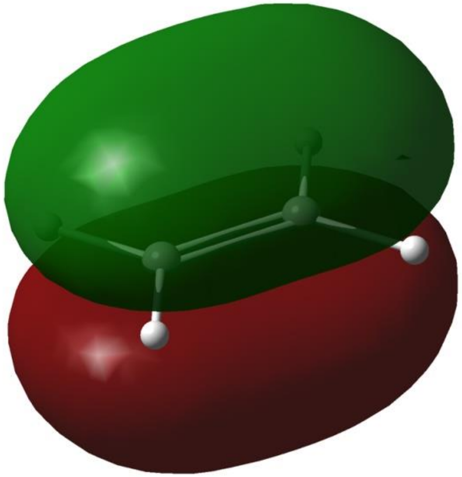
π-π*; λ_{max}=218

ε=11,000

n-π*; λ_{max}=320

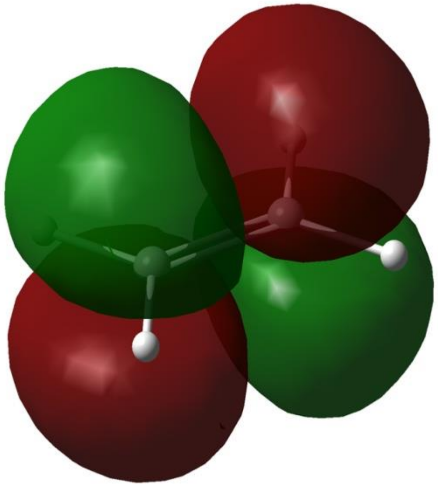
ε=100

Example: π → π* transitions responsible for ethylene UV absorption at ~170 nm calculated with ZINDO semi-empirical excited-states methods (Gaussian 03W):



HOMO π_u bonding molecular orbital

hν 170nm photon →



LUMO π_g antibonding molecular orbital

Possible Electronic Transitions 2

- HOMO: highest occupied molecular orbital
- LUMO: lowest unoccupied molecular orbital

- $\sigma \rightarrow \sigma^*$ and $\sigma \rightarrow \pi^*$ transitions: high-energy, accessible in vacuum UV (λ_{max} <150 nm). Not usually observed in molecular UV-Vis.
- $n \rightarrow \sigma^*$ and $\pi \rightarrow \sigma^*$ transitions: non-bonding electrons (lone pairs), wavelength (λ_{max}) in the 150-250 nm region.

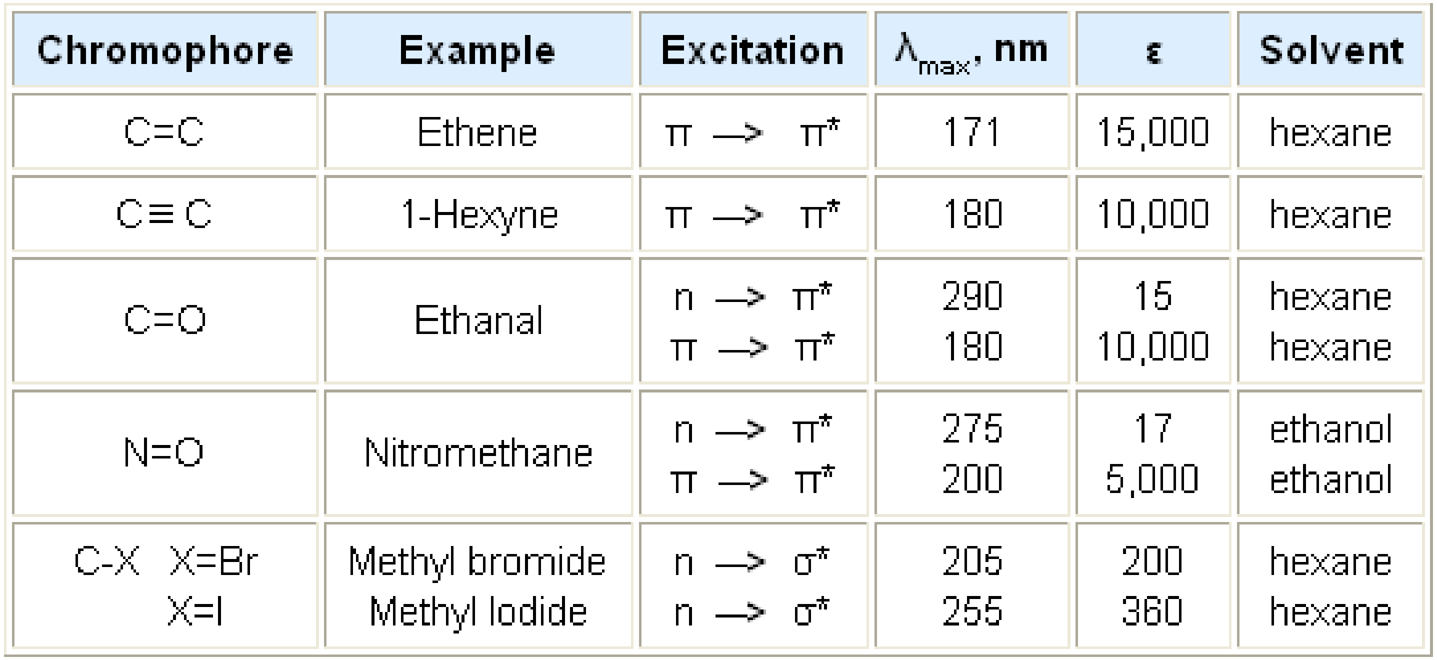
Possible Electronic Transitions 3

$n \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$

Related to most absorption of organic compounds

Experimentally convenient region of the spectrum (200 - 700 nm).

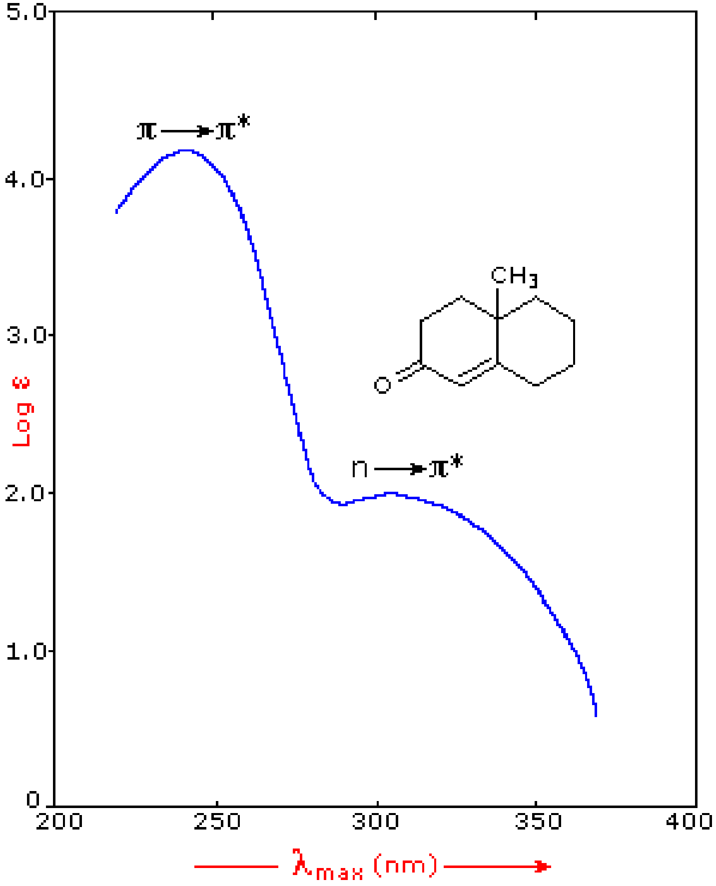
Molar absorption coefficient from $n \rightarrow \pi^*$ transitions are low (10 to100 L mol^{-1} cm^{-1}), but that from $\pi \rightarrow \pi^*$ transitions are between 1000 to10000 L mol^{-1} cm^{-1}).

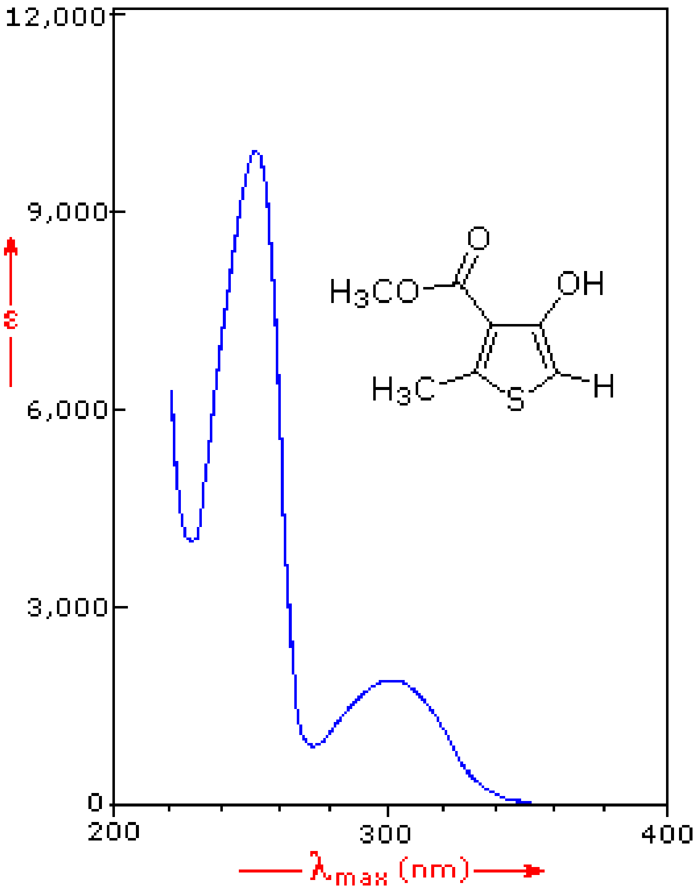


Chromophore	Example	Excitation	λ_{max}, nm	ε	Solvent
C=C	Ethene	$\pi \rightarrow \pi^*$	171	15,000	hexane
C≡C	1-Hexyne	$\pi \rightarrow \pi^*$	180	10,000	hexane
C=O	Ethanal	$n \rightarrow \pi^*$ $\pi \rightarrow \pi^*$	290 180	15 10,000	hexane hexane
N=O	Nitromethane	$n \rightarrow \pi^*$ $\pi \rightarrow \pi^*$	275 200	17 5,000	ethanol ethanol
C-X X=Br X=I	Methyl bromide Methyl Iodide	$n \rightarrow \sigma^*$ $n \rightarrow \sigma^*$	205 255	200 360	hexane hexane

$\pi \rightarrow \pi^*$ vs $n \rightarrow \pi^*$ transitions

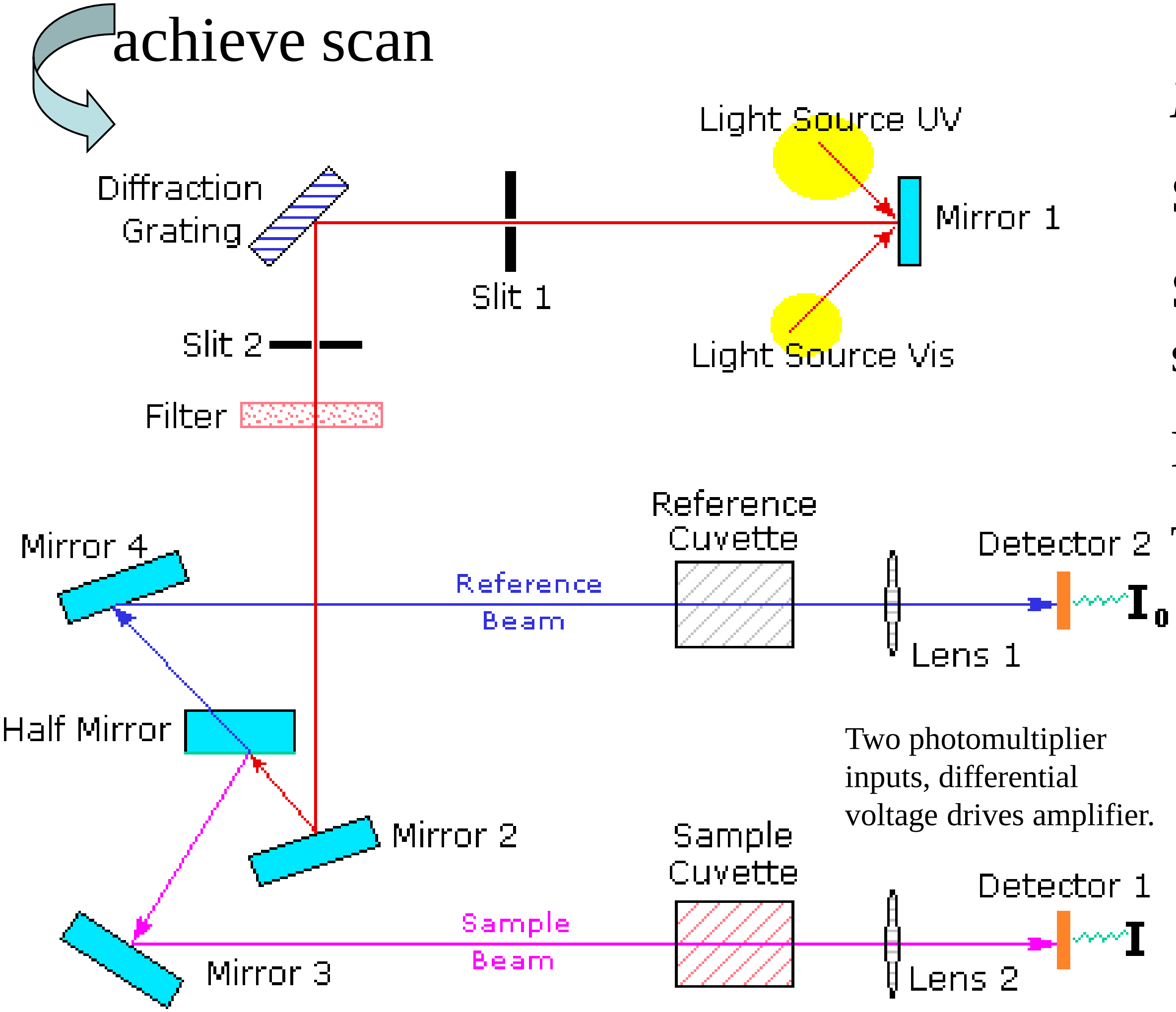
In general, the intensities from $\pi \rightarrow \pi^*$ transitions are stronger than those for $n \rightarrow \pi^*$ transitions





How Do UV Spectrometers Work?

Rotates, to achieve scan



Two photomultiplier inputs, differential voltage drives amplifier.

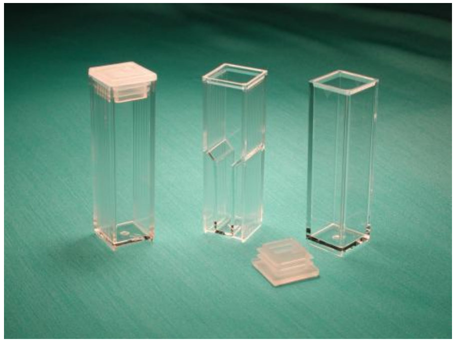
Matched quartz cuvettes

Sample in solution at ca. 10^{-5} M.

System protects PM tube from stray light

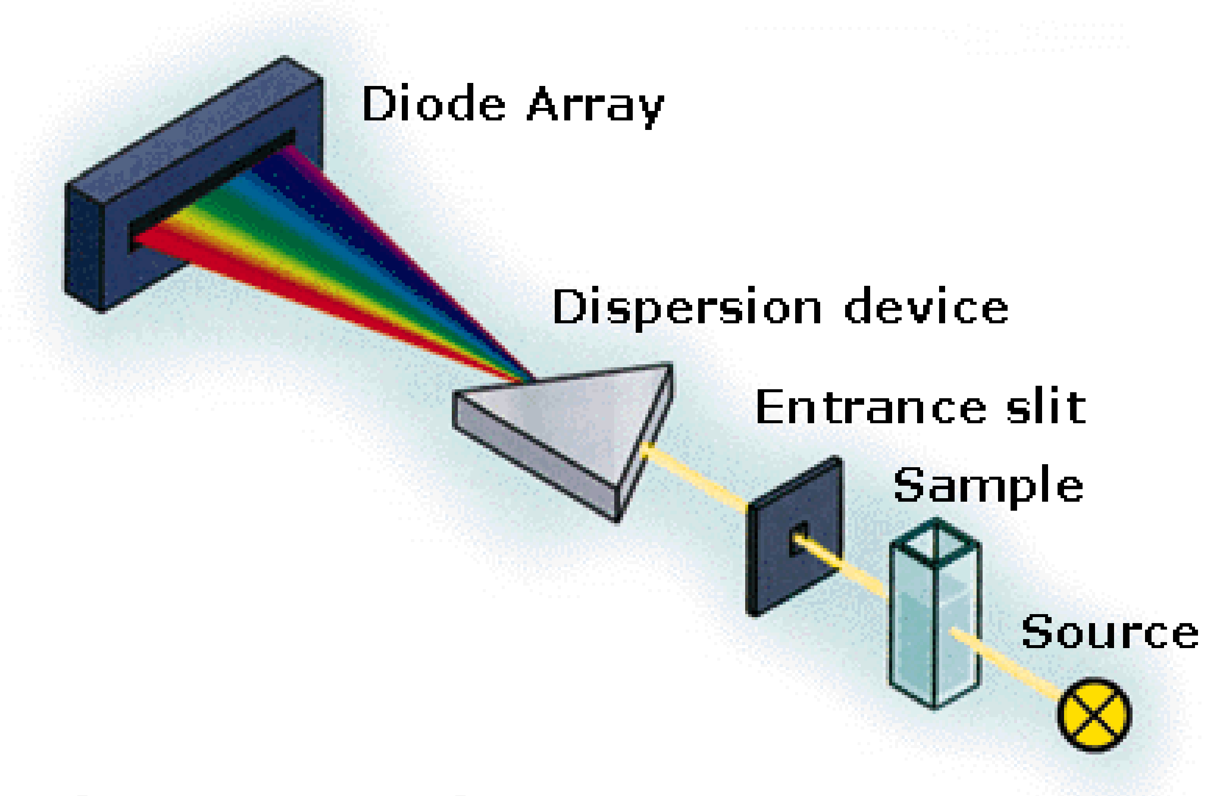
D_2 lamp-UV

Tungsten lamp-Vis



Diode Array Detectors

Diode array: array of photosensitive semiconductors after the light goes through the sample.

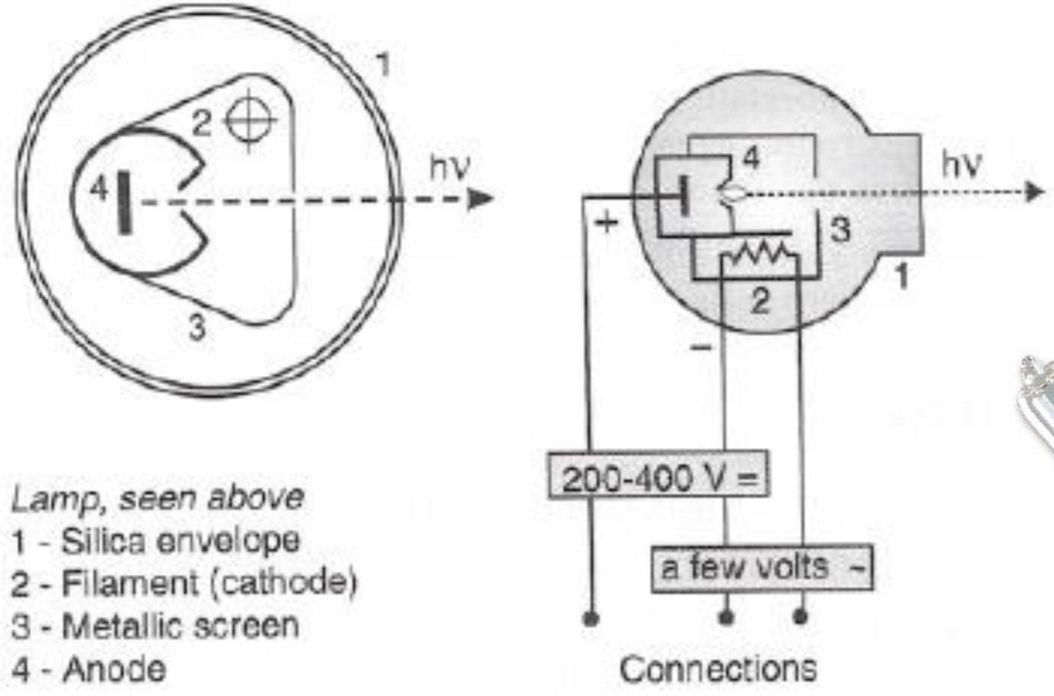


Advantage: speed, sensitivity,

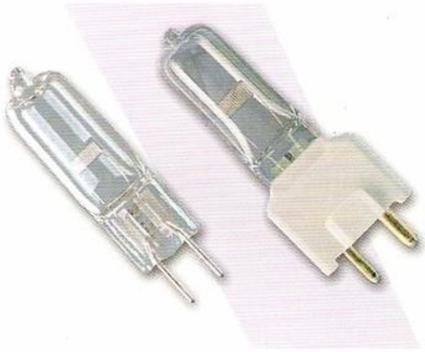
Disadvantage: resolution is 1 nm, vs 0.1 nm for normal UV

UV/Vis Source

$$D_2 + E_e \rightarrow D_2^* \rightarrow D' + D'' + h\nu \quad \text{(UV-Vis)}$$



Lamp, seen above
1 - Silica envelope
2 - Filament (cathode)
3 - Metallic screen
4 - Anode



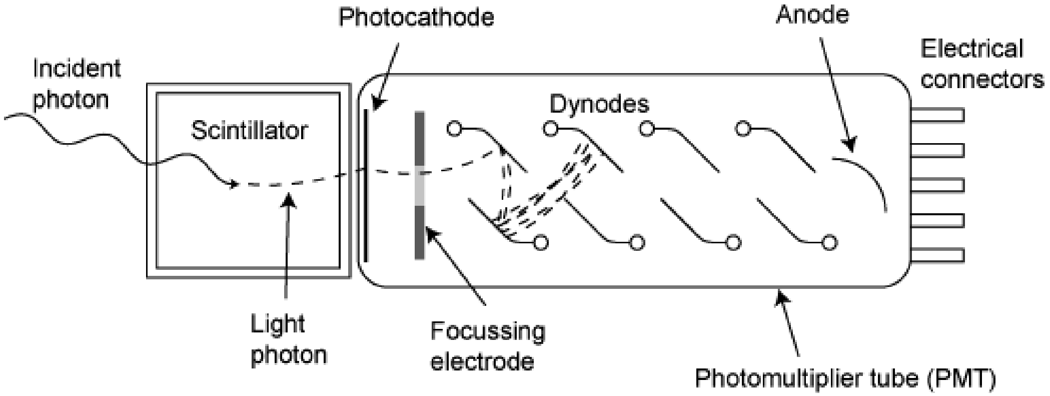
Halogen lamp
(Vis)

Deuterium arc lamp

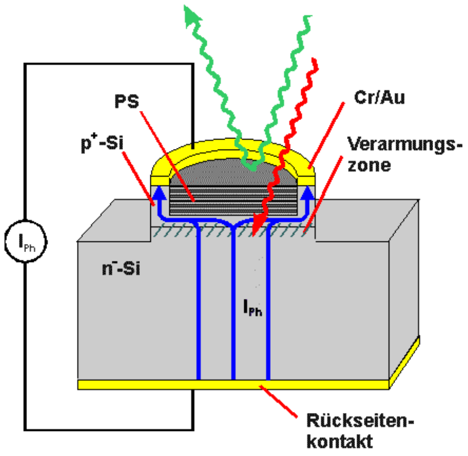
Light source change 360 - 300 nm

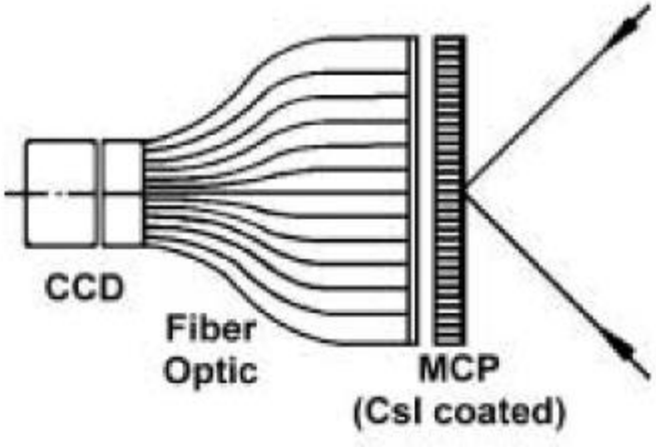
Detectors

Photomultiplier Tube (PMT): Single Channel



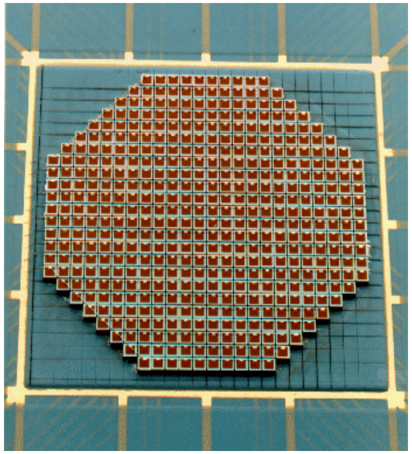
Photodiode Array: Multi-channel





One-dimensional

Two-dimensional

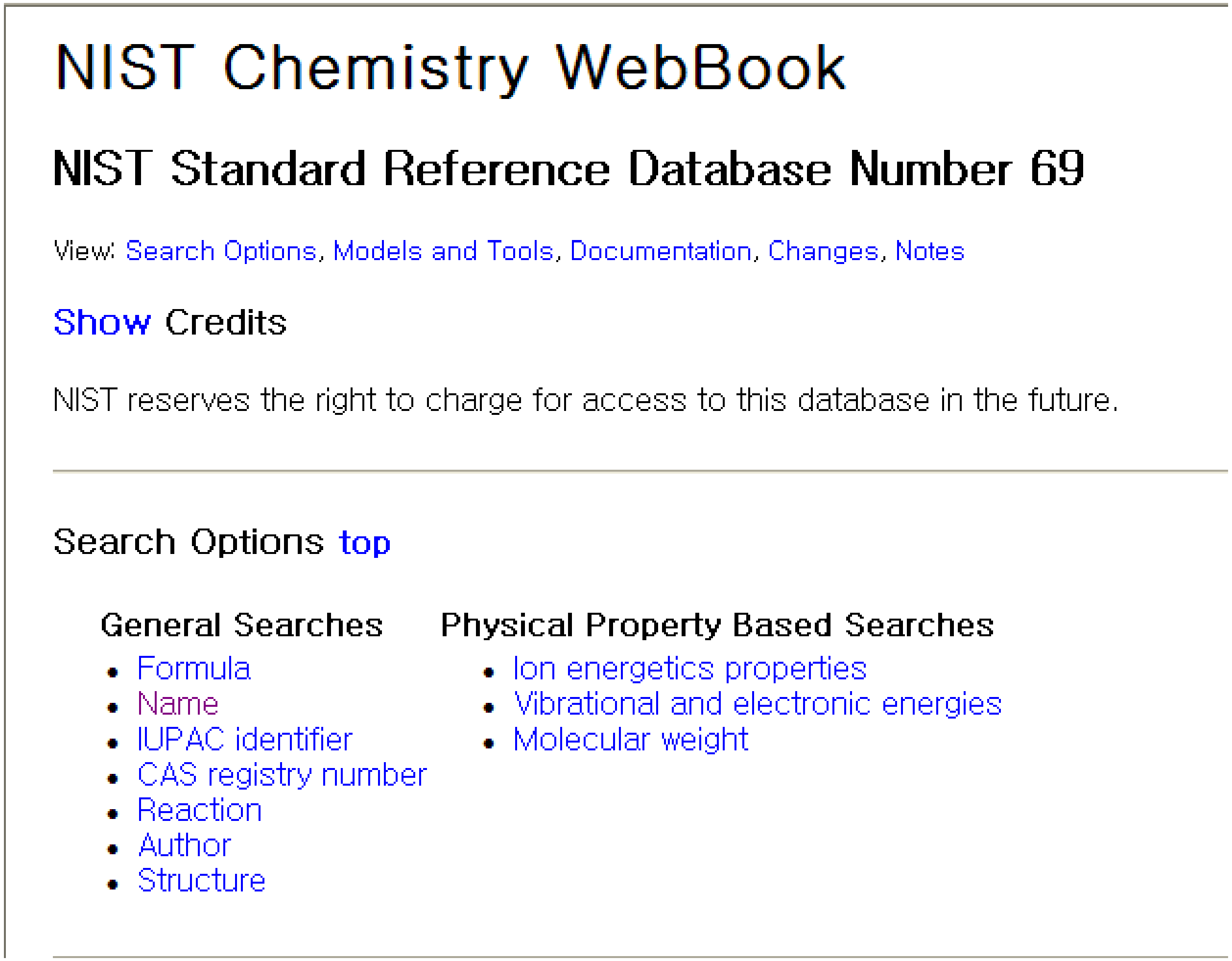


Experimental details

- What compounds show UV spectra?
  1. Generally think of any *unsaturated compounds* as good candidates. Conjugated double bonds are strong absorbers
  2. Just heteroatoms are not enough but C=O are reliable
  3. Most compounds have “end absorbance” at lower frequency. Unfortunately solvent cutoffs preclude observation.
  4. You will find molar absorbtivities ε in L•cm/mol, tabulated.
- Transition metal complexes, inorganics
- Solvent must be UV grade (great sensitivity to impurities with double bonds)
- The NIST databases have UV spectra for many compounds

NIST Chemistry WebBook

http://webbook.nist.gov/chemistry/



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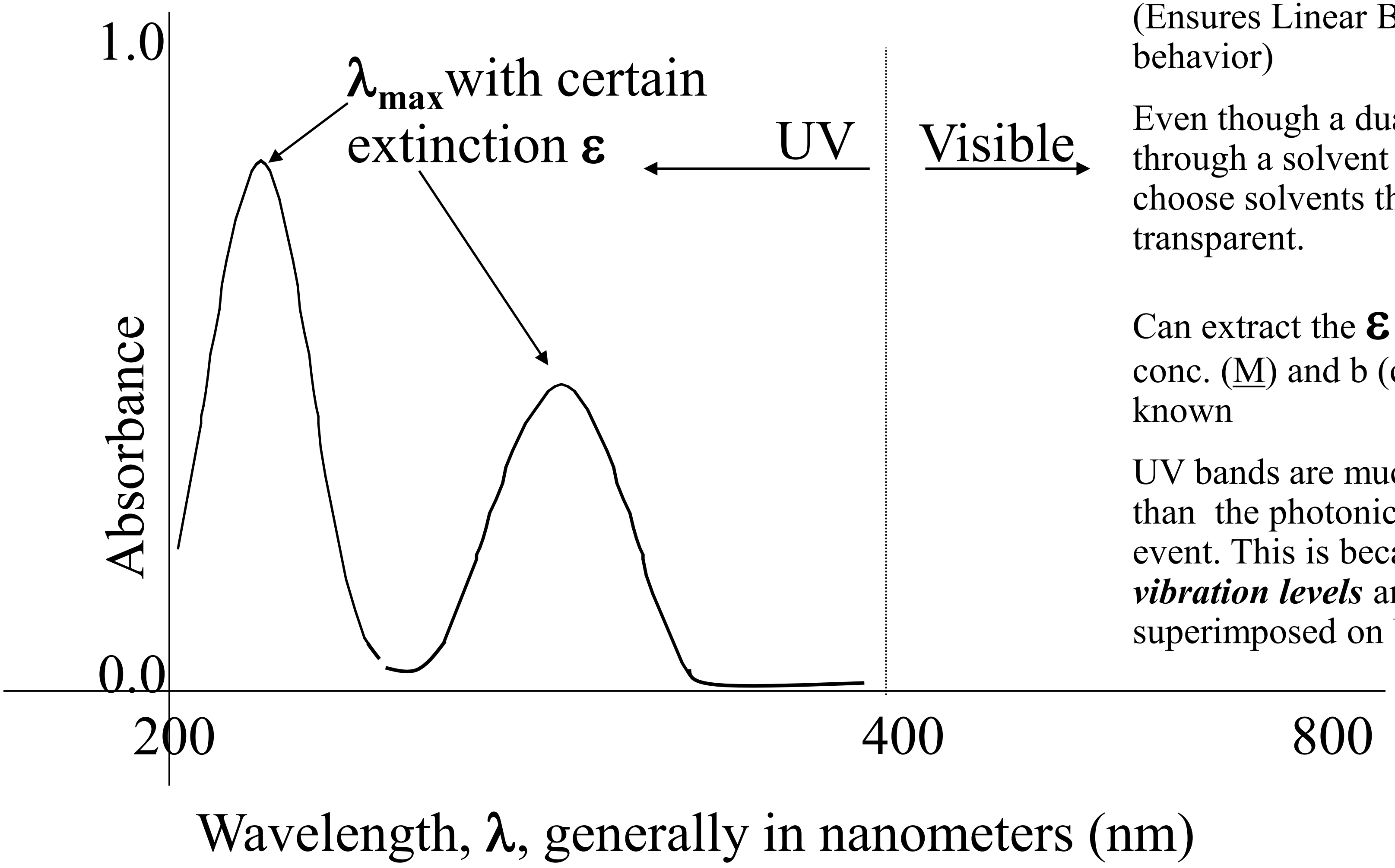
An Electronic Spectrum



Make solution of concentration low enough that **A≤ 1**

(Ensures Linear Beer's law behavior)

Even though a dual beam goes through a solvent blank, choose solvents that are UV transparent.

Can extract the **ε** value if conc. (M) and b (cm) are known

UV bands are much broader than the photonic transition event. This is because ***vibration levels*** are superimposed on UV.

An Electronic Spectrum

- Some solvents absorb UV at lower wavelength

- For quantitative work, the cut-off may be set at a wavelength (λ_0) where the absorbance for 10 mm pathlength of the solvent exceeds 0.05 absorbance units (relative to water)

Solvents for UV (Showing High Energy Cutoffs)

Water	205
$CH_3C\equiv N$	210
C_6H_{12}	210
Ether	210
EtOH	210
Hexane	210
MeOH	210
Dioxane	220
THF	220
CH_2Cl_2	235
$CHCl_3$	245
CCl_4	265
benzene	280
Acetone	300

Various buffers for HPLC, check before using.

Organic compounds (many of them) have UV spectra

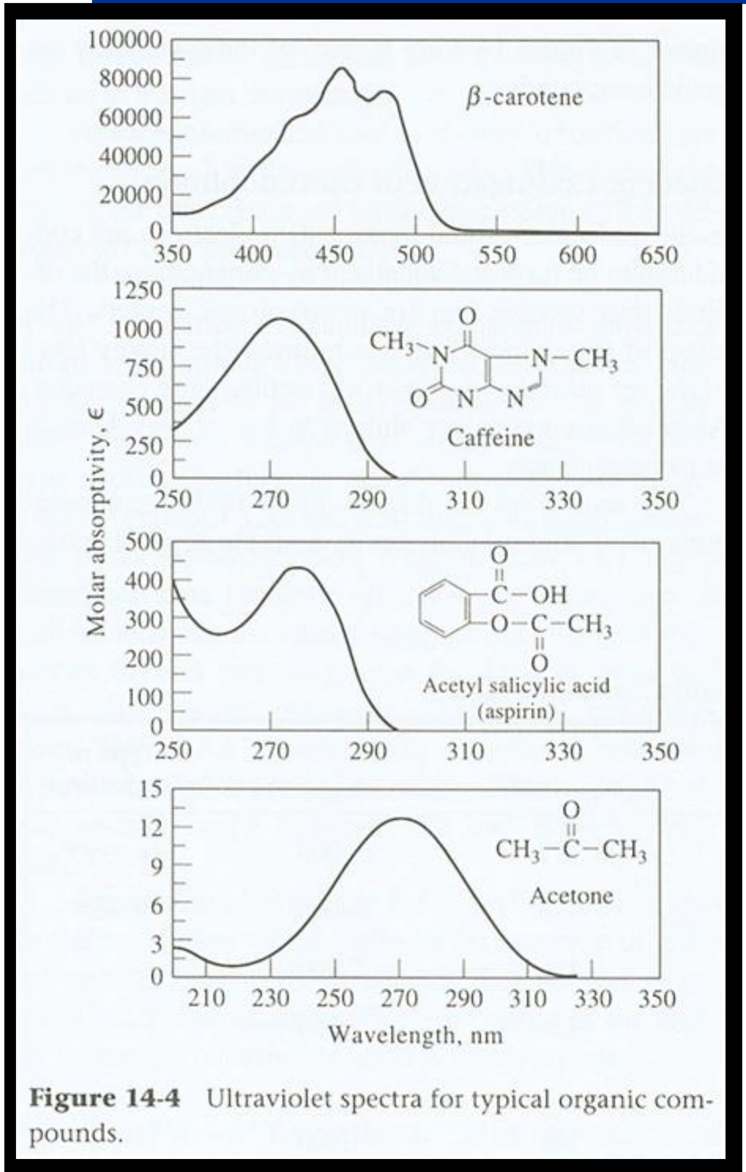
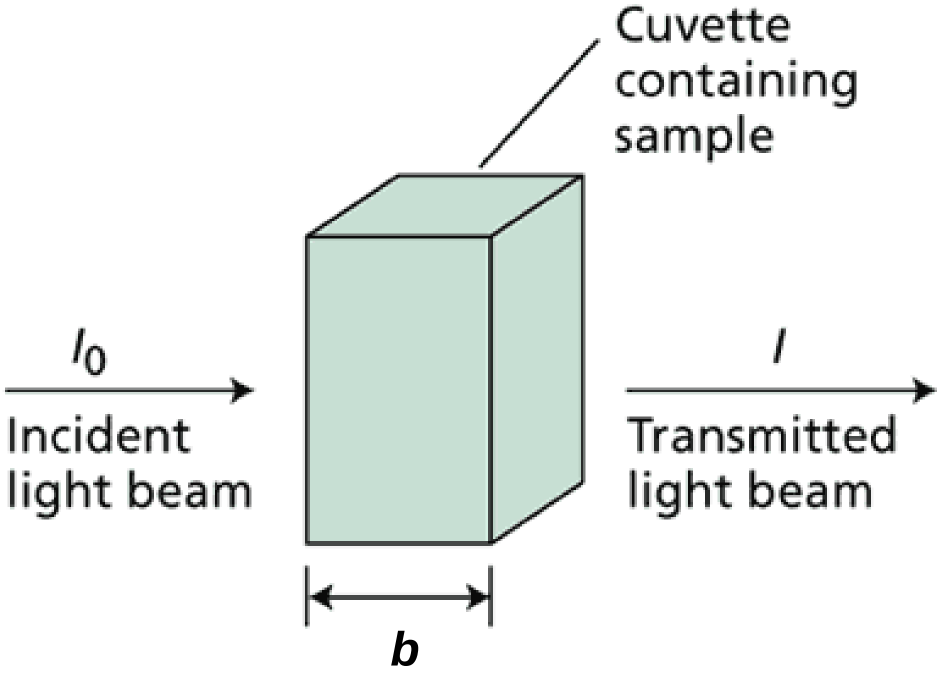
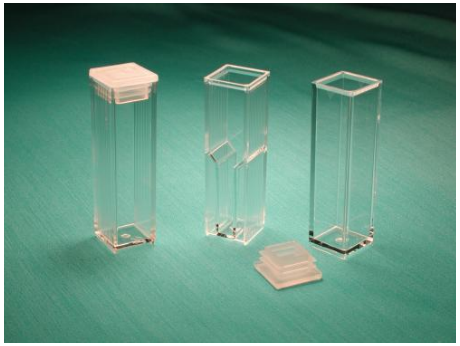


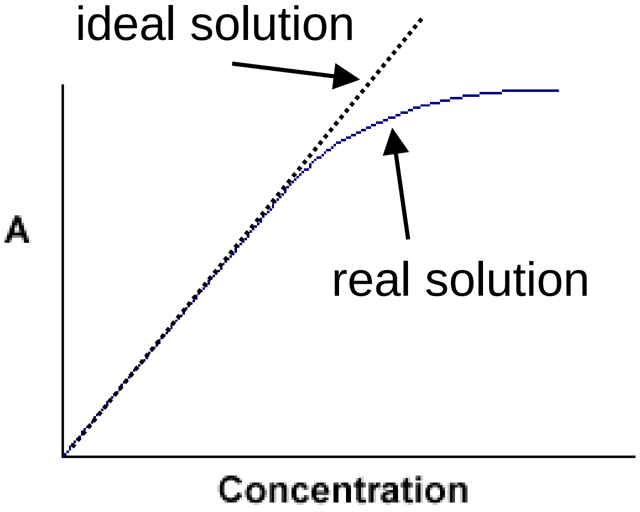
Figure 14-4 Ultraviolet spectra for typical organic compounds.

- UVs can be very non-specific
- Its hard to interpret except at a cursory level, and to say that the spectrum is consistent with the structure
- Each band can be a superposition of many transitions
- Generally we don’t assign the particular transitions.

Beer's (Beer-Bouger-Lambert) Law







Absorbance (A) = $-\log(I/I_o)$

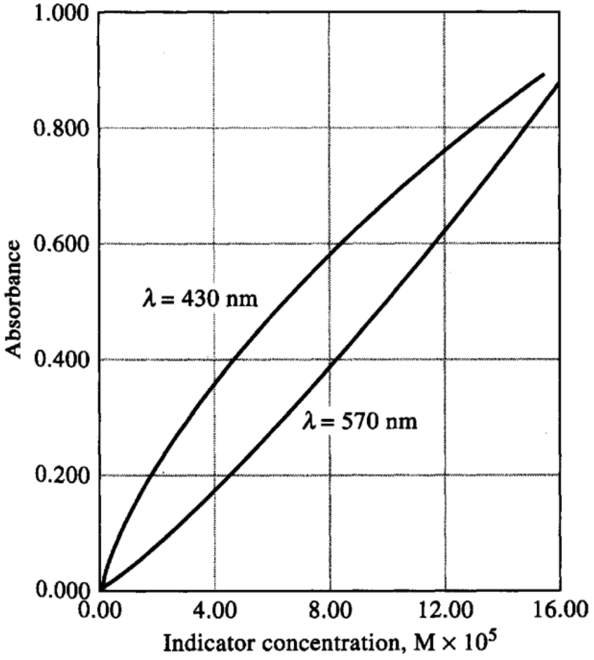
$A = \varepsilon bC$, ε = *molar absorptivity* ($cm^{-1}M^{-1}$)

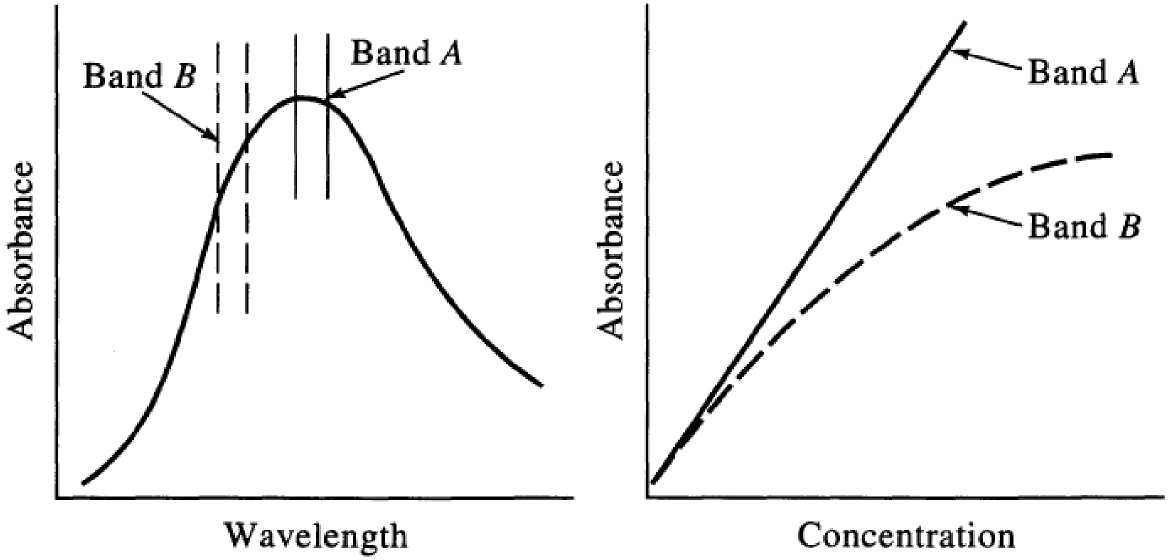
Beer’s (Beer-Bouger-Lambert) Law

1. Linear absorbance (A) with increased concentration-directly proportional
2. Makes UV useful for <u>quantitative analysis</u> and in <u>HPLC detectors</u>
3. Above a certain concentration the linearity curves down, loses direct proportionality
4. Recommended $A_{\text{max}} < 1.0$

Limitations of Beer’s Law

1. Chemical effects - analyte associates, dissociates or reacts to give molecule with different ε
2. Physical effects - stray light, polychromatic radiation or noise
    - As concentration increases there will be a consequent change in refractive index, *n.*
    - As concentration increases the solute begins to have a significant influence on the local electromagnetic environment.

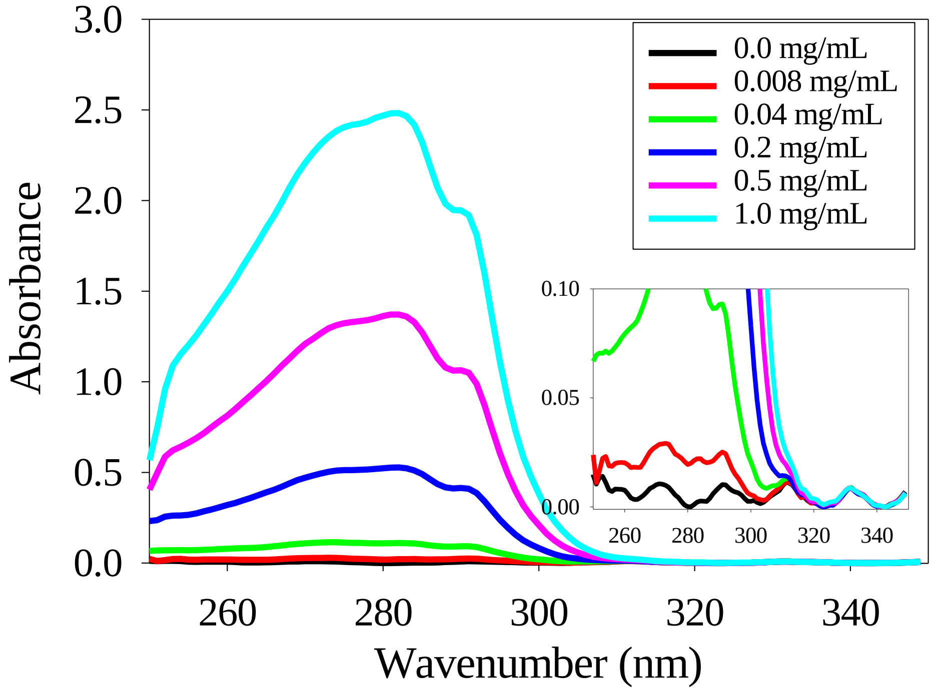




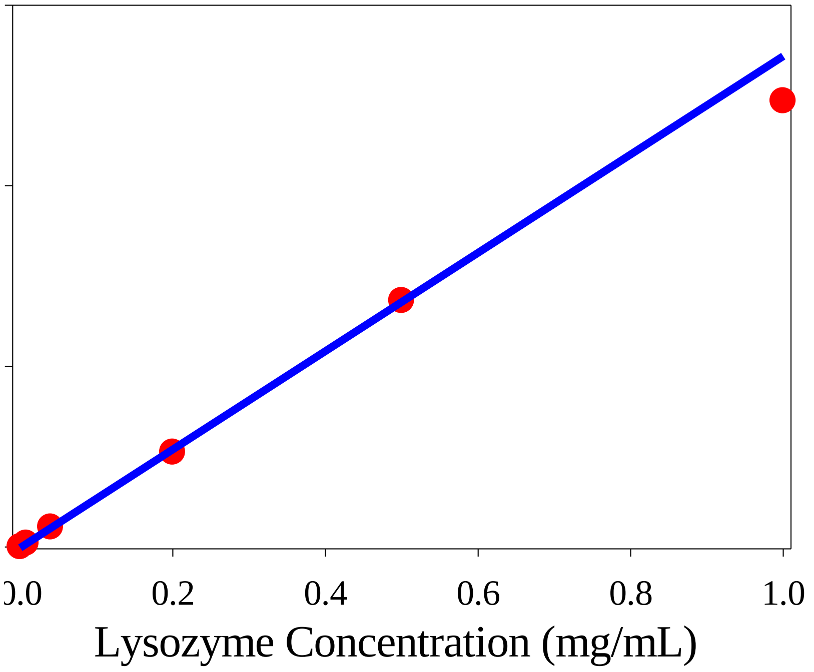
Application of Beer’s Law

Determination of concentration of unknown compounds

UV Spectra of Lysozyme

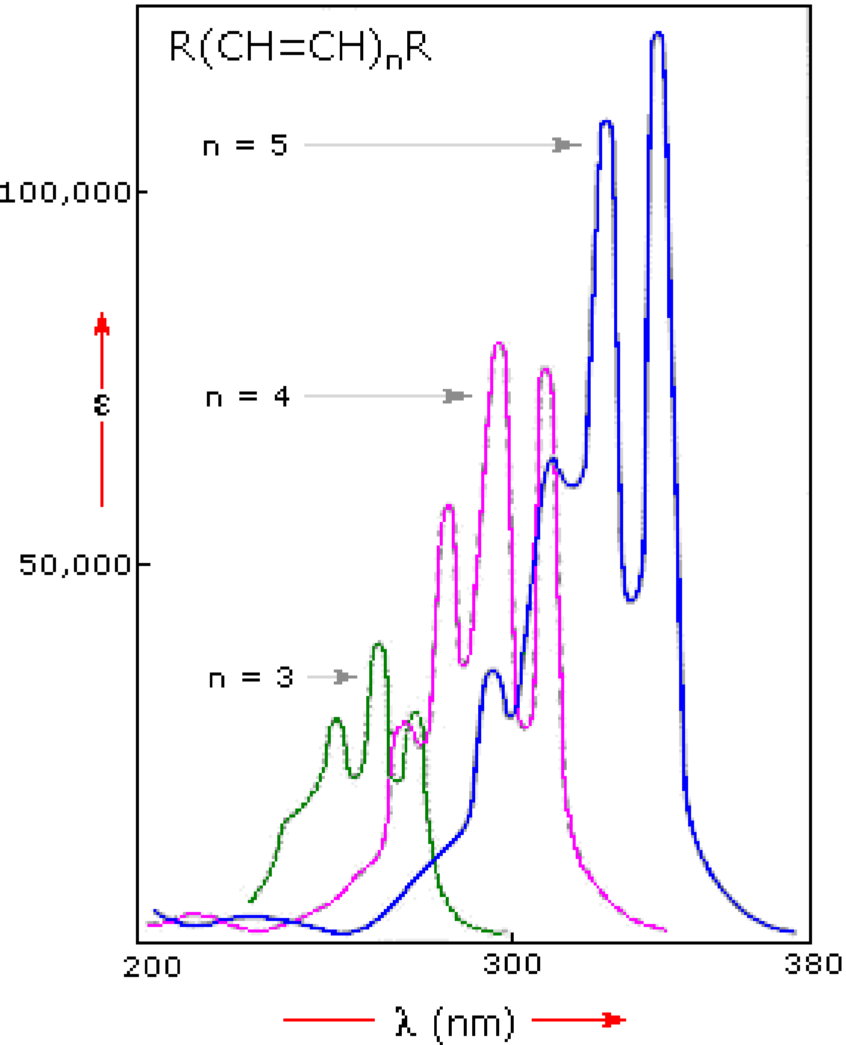


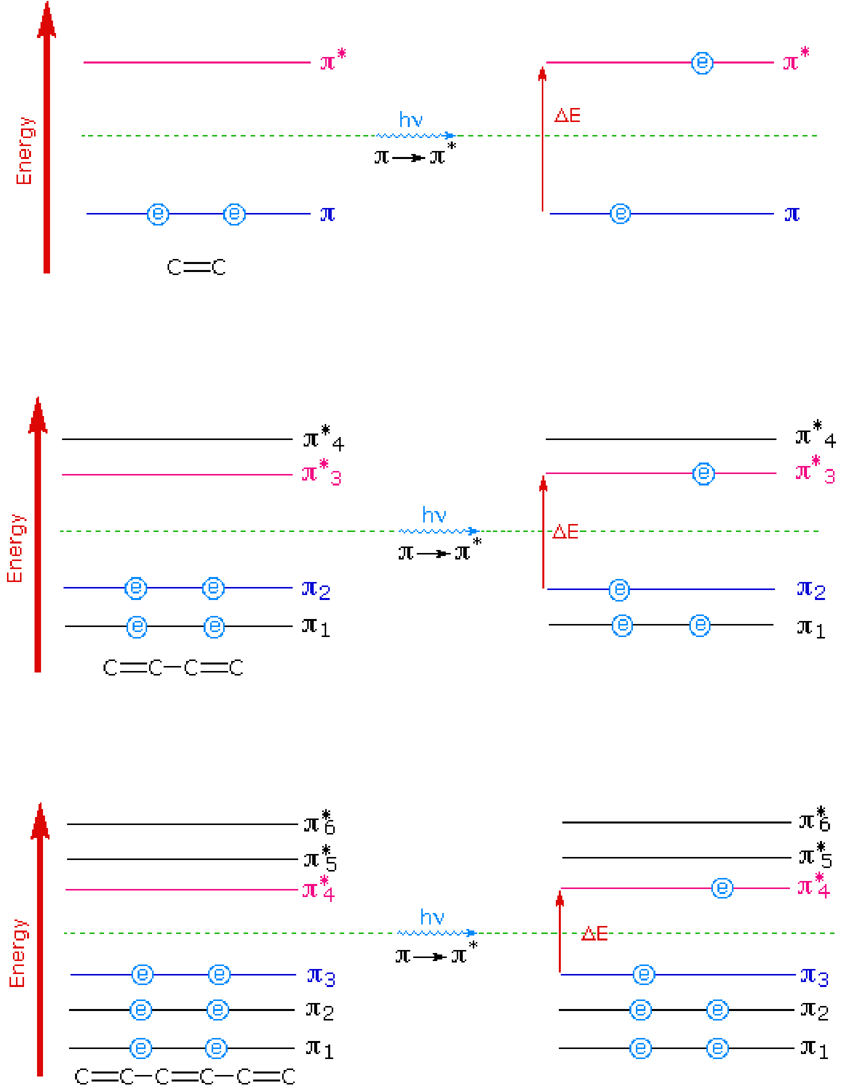
Absorbance vs. Concentration



Conjugation

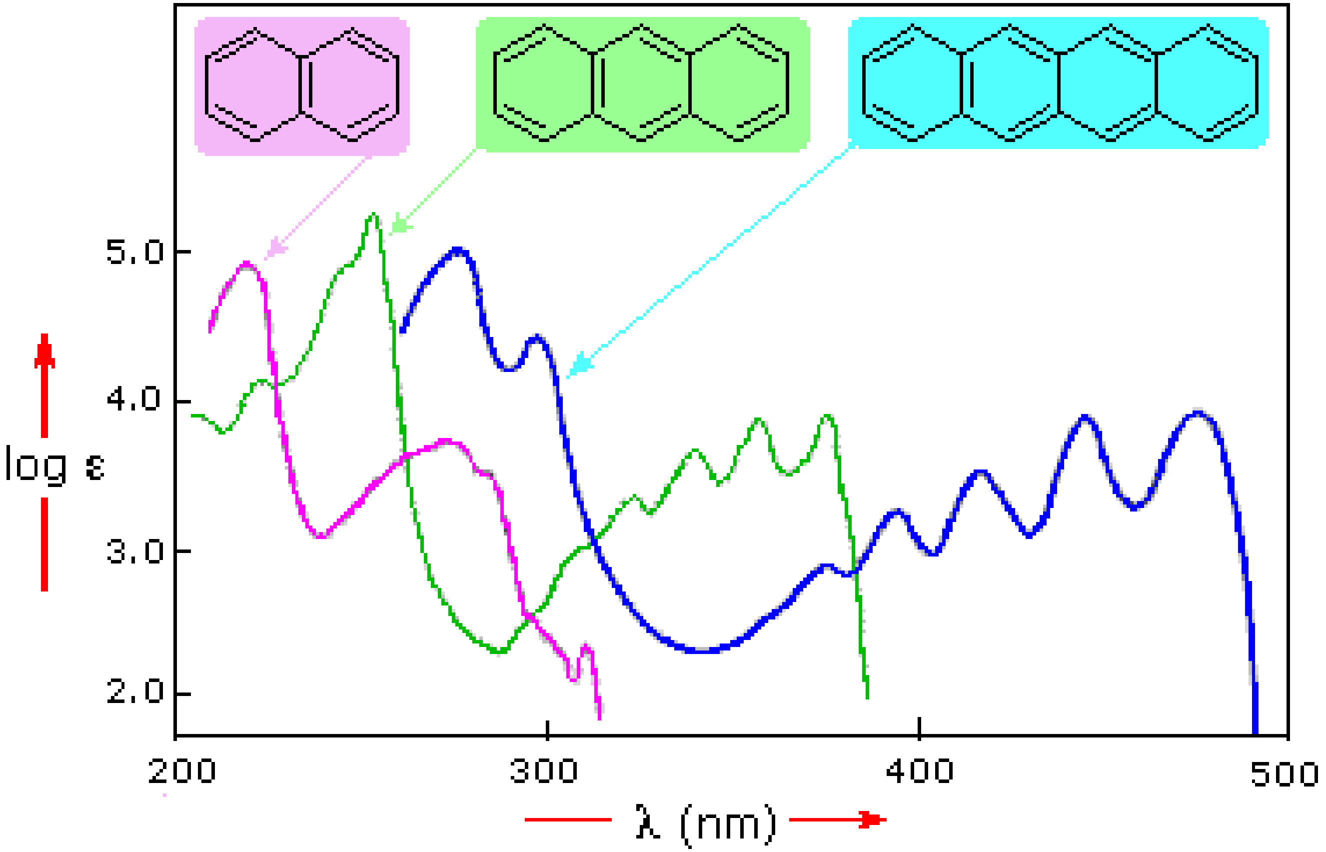
More conjugated π systems, more π orbitals, and the energy gap between HOMO and LUMO is decreased (200 – 800 nm)





Polycyclics

The added conjugation in naphthalene, anthracene and tetracene causes bathochromic shifts (red shifts) of these absorption bands, as displayed in the chart on the left below.



Conjugation with Lone Pair Electrons

For unsaturated aldehydes and ketones and aromatic ring compounds, the π → π* absorption located at 242 nm is very strong, with an ε = 18,000. The weak n → π* absorption near 300 nm has an ε = 100.