Optical Spectroscopy

Ultraviolet-Visible

Interaction of matter (molecules) with electromagnetic radiation ~ optical spectroscopy.

Basic facts of electromagnetic radiation (wave)

– All electromagnetic radiation can be considered as waves

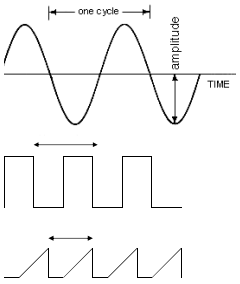
Characteristics of electromagnetic waves:
– Wavelength (λ), frequency (ν), wavenumber ($\bar{\nu}$) ('color' of light)
– wavenumber = $1/\lambda$ (number of waves / unit length)

Blue light ~ 400-450 nm wavelength, 25 000 – 22 222 cm^{-1}
Red light ~ 600-700 nm wavelength, 16 667 – 14 286 cm^{-1}

– Polarization: direction of transversal vibration
– linearly polarized light vs. randomly polarized
– Speed: in vacuum (n=1); c = 2.99×10^8 m/s

Considered as a particle: photon (energy packet) of energy $h\nu$.

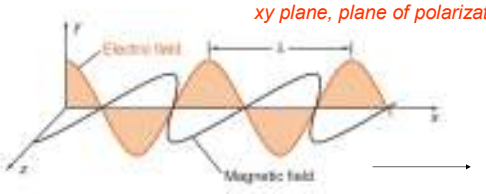
A wave a periodic change of a ***property*** in space and/or time.



Basic facts of electromagnetic radiation (wave)

EM radiation as a *travelling* wave; has mutually perpendicular magnetic and electrical fields and these fields vary periodically in *time and space*.

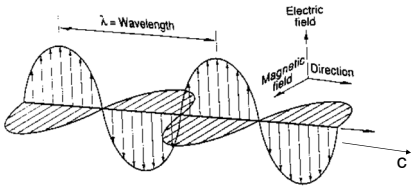
xy plane, plane of polarization



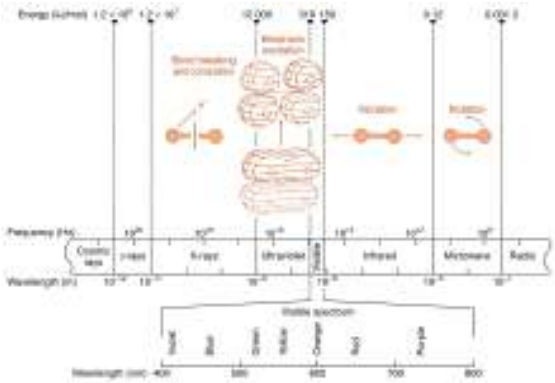
Instantaneous snap shot of a linearly polarized wave with graphic of field intensities.

$$\text{photon energy} = h\nu = \frac{hc}{\lambda}$$

Distance between two consecutive similar points along the wave (with same phase and amplitude) is λ.



$\lambda\nu = c$

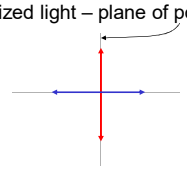


Electromagnetic radiation covers a large energy range

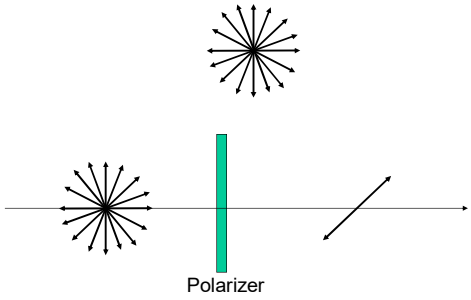
Animation of a pencil of polarized light.

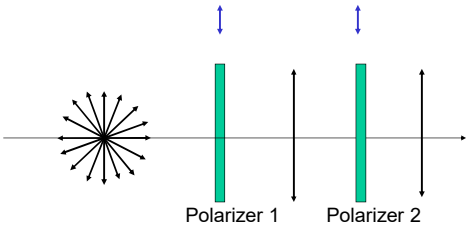
Polarization of light: electromagnetic wave is a transverse wave which has both an electric and a magnetic component.

View a polarized electromagnetic wave traveling towards you, Then you would observe the amplitude variation of the wave in one plane – polarized light – plane of polarization.

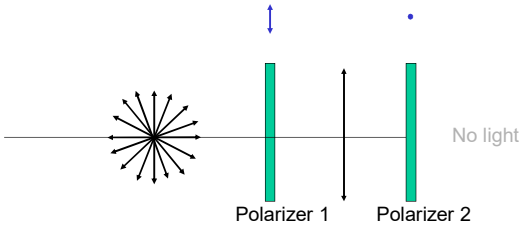


Natural light: consists of pencils of light with planes of polarization in *all possible directions*.





Polarizers:
Planes of polarization parallel



Polarizers:
crossed

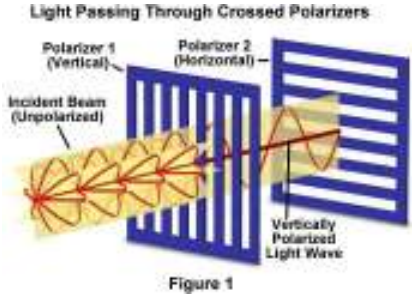


Figure 1

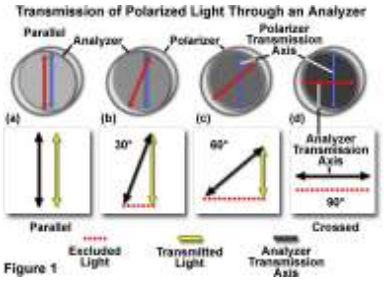
http://www.olympusmicro.com/primer/lightandcolor/polarization.html

Polarization of Light

http://www.olympusmicro.com/primer/java/polarizedlight/filters/index.html

Polarization of Light (3-D Version)

http://www.olympusmicro.com/primer/java/polarizedlight/3dpolarized/index.html



http://www.olympusmicro.com/primer/java/polarizedlight/filters/index.html

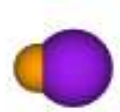
The energy (E) of a molecule consists of translational, rotational, vibrational and electronic energy (major terms).

$E_{molecule}$ =
$E_{nuclearspin} + E_{electronspin} + E_{translational} + E_{rotation} + E_{vibration} + E_{electronic}$

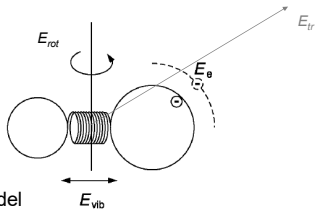
Translation – *movement of the molecule through space*
Spin – *nuclear and electron spin*
Rotational – *rotation of the molecule*
Vibrational – *vibration of the atoms with respect to each other*
Electronic – *mutual interactions of the electrons and nuclei*

All these energy states (stationary states) are quantized except translational energy.

Quantized states have precisely assigned energy values.



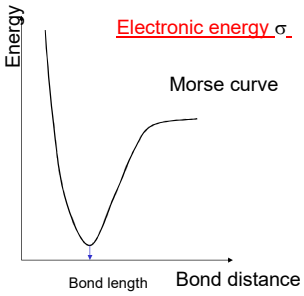
diatomic molecule



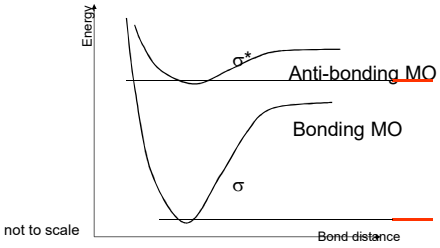
model

In molecules electrons reside molecular orbitals, σ, π, σ*, π* and non-bonding orbitals

Morse curve shape depend on the molecular geometry of the state.

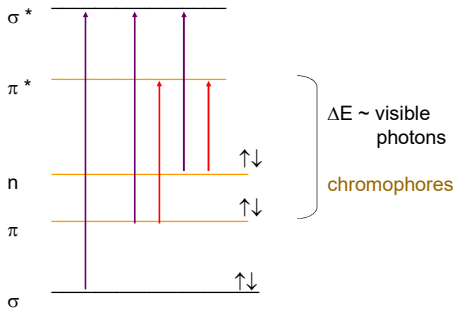


For any covalent bonding orbital in a molecule there is a corresponding anti-bonding orbital of higher energy.



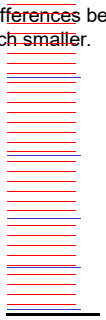
The difference in energy between molecular bonding, non-bonding and anti-bonding orbitals ranges from 125-650 kJ/mole

Electronic energy levels and transitions



Each electronic state is associated with a set of vibrational states; and each vibrational state is associated with a set of rotational states.

The energy differences between rotational and vibrational levels are much smaller.

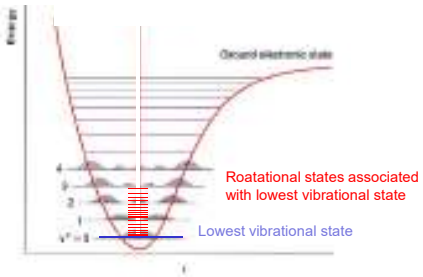


Electronic level

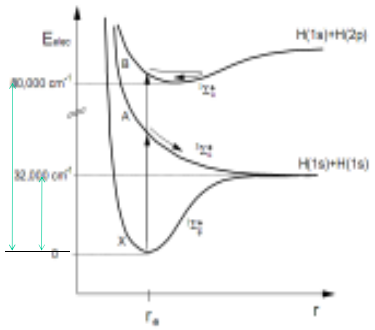
Vibrational levels

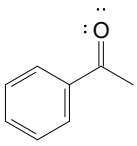
Rotational levels

Relative energy values in different domains



$E_{molecule} = E_{rotation} + E_{vibration} + E_{electronic}$



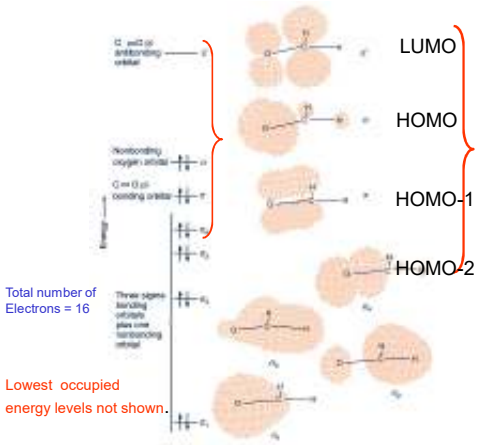


Covalent molecules: Bonding involves molecular orbitals.

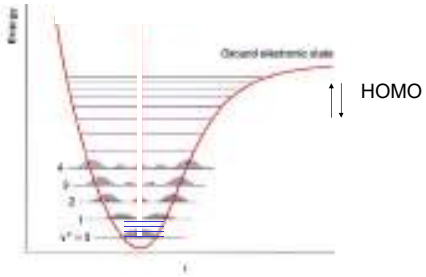
Electrons are in molecular orbitals σ,π,δ,..and lone pairs (generally many molecular orbitals exist, most important here HOMO and LUMO).

In addition σ*,π*,δ*,..(anti-bonding molecular orbitals are formed).

Most molecules in ground state have electron spins paired, no net spin, singlet states.



Relative energy values in different domains: *ground state HOMO orbital*



$E_{molecule} = +E_{rotation} +E_{vibration} +E_{electronic}$

Ground State Molecular Orbitals of molecules: Chromophores

A functional group capable of having characteristic electronic transitions (>200nm) is called a chromophore.

σ bonding

π bonding

Non-bonding

Photon and molecule interactions

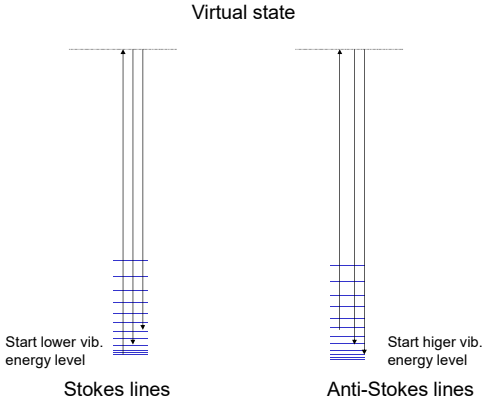
When electromagnetic radiation (photon) 'interacts' with a molecule, scattering or absorption occurs at the outset.

Types of scattering:

Elastic – all energy of the colliding photon is preserved, wavelength of light remains unaltered (Rayleigh scattering – elastic scattering).

Stokes: Raman – some energy is lost as vibrational energy of The molecule, emitted photon wavelength is longer than the original wavelength of the photon. Inelastic scattering.

Anti-Stokes: Raman – emitted photon has shorter wavelength than the original. Inelastic scattering.



Photon and molecule interactions: Absorption

In an absorption event, a single photon of light is fully absorbed by a single molecule; energy of the molecule is increased by the energy of the photon.

$$\Delta E = h\nu$$

h = Plank's constant

– The high energy molecule relaxes by processes:
  i. non-radiative – heat
  ii. radiative – fluorescence
  – phosphorescence
  _ stimulated emission

– leads to changes in molecular structure/chemical reaction – e.g. *Photosynthesis*

UV-VIS Spectroscopy

Photons in the region of UV-VIS are of energy comparable to energy required for transitions between electronic states.

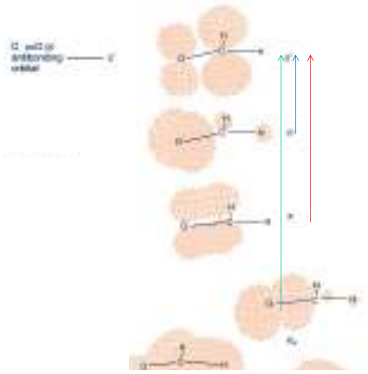
UV-VIS absorption arises from electronic transitions.

Atoms → molecules : atomic orbitals → molecular orbitals
Electronic transitions ↔ molecular orbitals.

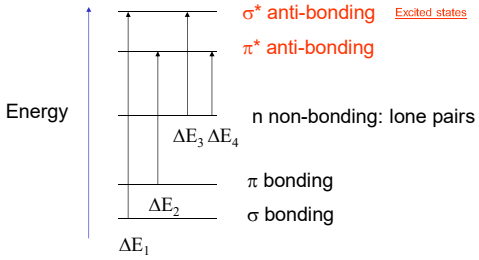
Ions, complexes → d-d transitions and/or charge transfer complexes (internal redox reaction)

The difference in energy between bonding, non-bonding and anti-bonding orbitals ranges from 125-650 kJ/mol; corresponds to EM radiation in UV 100-350nm, and VIS 350-700nm.

Light absorption changes the charge distribution of electrons of the molecule.



Absorption in UV-VIS Region
General electronic energy hierarchy in molecules



$\Delta E_1 > \Delta E_2 > \Delta E_3 > \Delta E_4$

Some transitions

Chromophores


σ → σ* 135nm

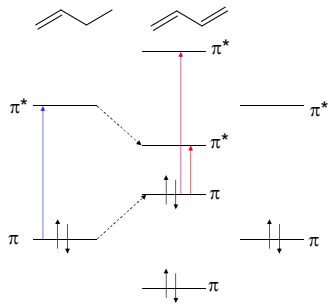

σ→ σ* 150nm
n → σ* 183nm


π → π* 175nm

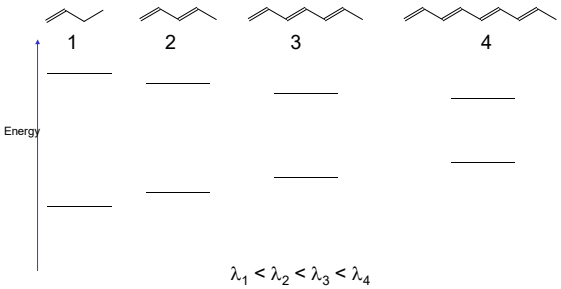

π → π* 254nm


n → π* 290nm

Conjugation: Spreading of π-MOs over the molecular framework. Leads to *narrowing* of HOMO-LUMO gap. Increases λ_{max} - bathochromic effect.



Conjugation: Spreading of π-MOs over the molecular framework. Leads to narrowing of HOMO-LUMO gap.

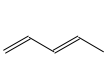


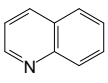
$\lambda_1 < \lambda_2 < \lambda_3 < \lambda_4$

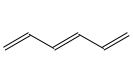
$$A_\lambda = \varepsilon_\lambda l c$$

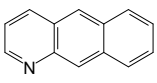

λ_{max}=180-200nm
ε=10000

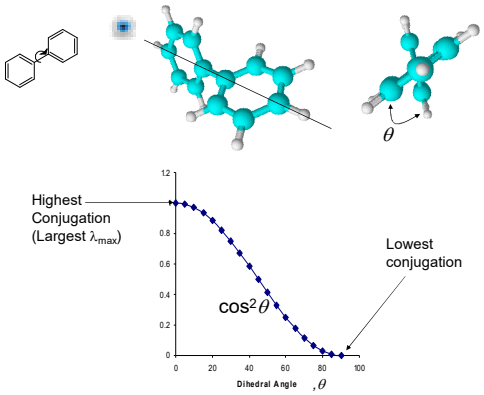

λ_{max}=250nm


λ_{max}=217nm
ε=21000

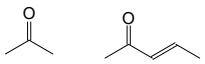

λ_{max}=290nm


λ_{max}=258nm
ε=35000


λ_{max}=360nm

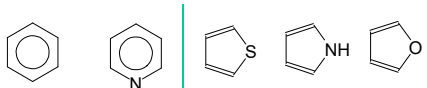


n-electron conjugation: n → π*

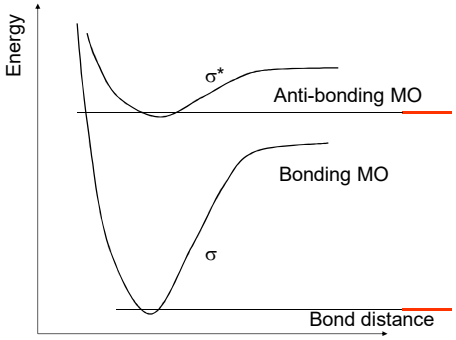


290 328

π-electron conjugation: π → π*



254 250 232 217 210

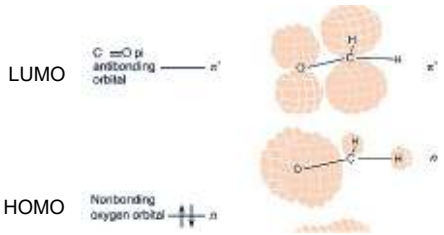


Photon energy, frequency and wavelength relationships

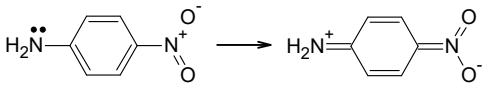
$\Delta E_1 > \Delta E_2 > \Delta E_3 > \Delta E_4$

$\nu_1 > \nu_2 > \nu_3 > \nu_4$

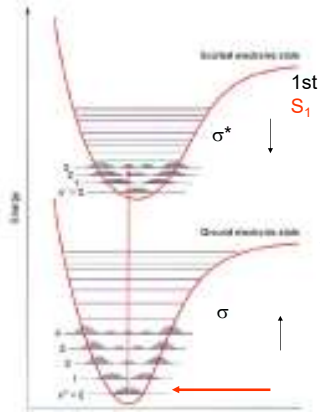
$\lambda_1 < \lambda_2 < \lambda_3 < \lambda_4$

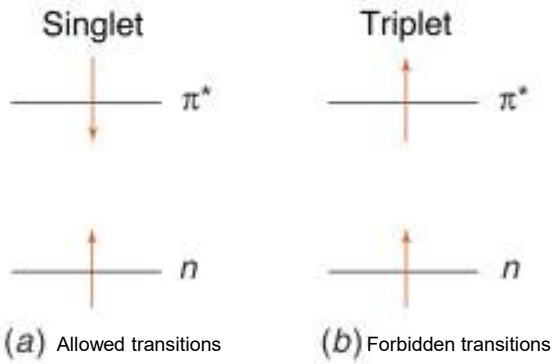


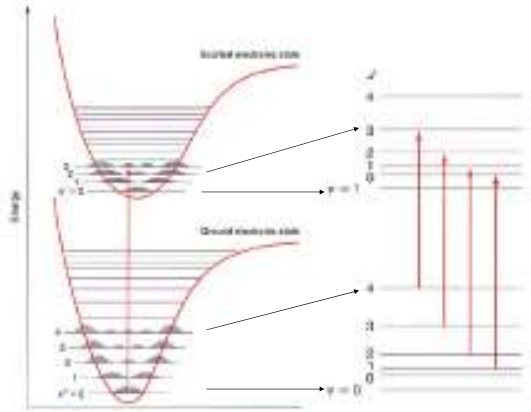
Intramolecular charge transfer transitions absorbs radiation in the electronic spectra.

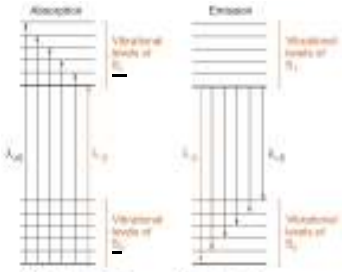


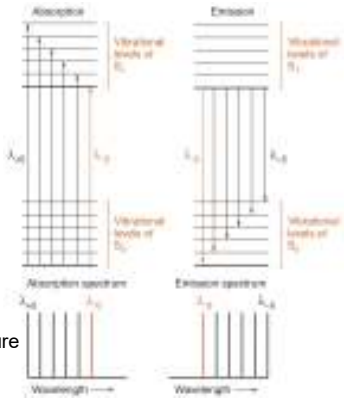
$$\nu = \frac{IE - EA}{h}$$



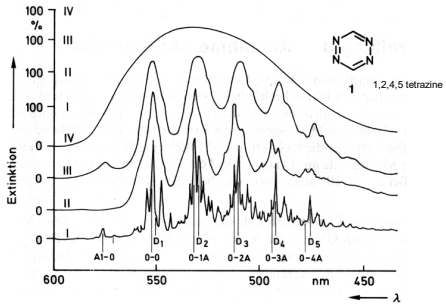








Fine structure



I Gas phase, room temperature

II In isopentane-methylcyclohexane matrix, 77K

III In cyclohexane, room temperature

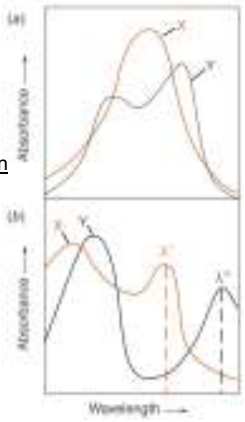
IV In water, room temperature

http://131.104.156.23/Lectures/CHEM_207/uv-vis.htm

Effect of the environment on the spectral shape.

Condensed phase spectra do not have much fine structure due to significant line broadening.

Gas phase UV-VIS spectra has fine structure.



Lambert-Beer Law (all absorption Spectroscopies)

$A_\lambda = \varepsilon_\lambda lc$

Absorbance (optical density)

ε_λ = molar absorptivity (extinction coefficient)

l = path length (cm)

c =concentration (M)

Lambert-Beer Law: Derivation

A beam of monochromatic light falling on an absorbing and homogeneous medium loses the intensity of the beam (Power, P) as it traverses through the medium. The loss of the power of the beam, dP, for a traveled path length, dx, is proportional to the concentration of the absorbing species, power of the beam and the length dx.

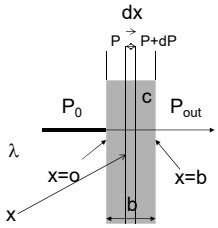
$dP \propto c$

$dP \propto dx$

$dP \propto P$

Therefore,

$dP = -\beta cPdx$



$dP \propto Pcdx$

$\frac{dP}{P} = -\beta cdx$

Integrating within limits;

$$\int \frac{dP}{P} = -\int \beta cdx \quad \Rightarrow \quad \int_{P_0}^{P_{out}} \frac{dP}{P} = -\int_0^b \beta cdx \quad \textit{which is} \quad \ln \frac{P_{out}}{P_0} = -\beta bc$$

$$2.303 \log_{10} \frac{P_{out}}{P_0} = -\beta bc \qquad \log_{10} \frac{P_0}{P_{out}} = \frac{\beta bc}{2.303} = \varepsilon bc \qquad A = \varepsilon bc$$

$A_\lambda = \varepsilon_\lambda bc$

↑ molar absorptivity at λ

Absorbance is directly proportional to c.

Other forms:

$$T = \frac{P_{out}}{P_0}$$

$$\%T = \frac{P_{out}}{P_0} \times 100$$

$$A = \log\left(\frac{P_0}{P_{out}}\right)$$

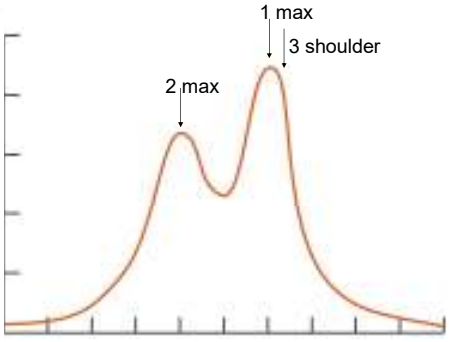
$$A = -\log_{10}(T) = \log_{10}\left(\frac{1}{T}\right)$$

molar absorptivities vary by orders of magnitude:

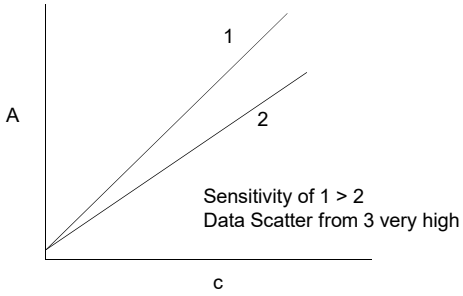
10^4-10^6 are termed *high intensity absorptions*

10^3-10^4 are termed *low intensity absorptions*

0 to 10^3 are the absorptions of *forbidden transitions*



Calibration curve:



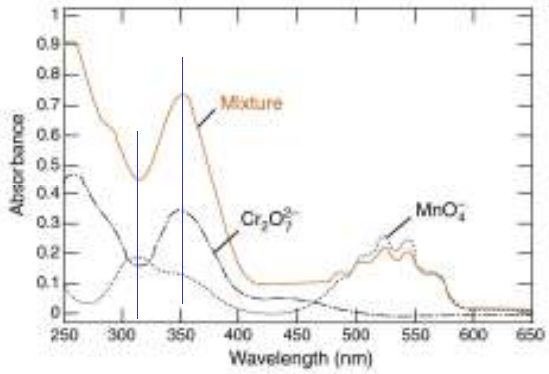
Absorption of a molecule is usually characterized by its molar absorptivity.

$A_\lambda = \varepsilon_\lambda bc$

ε_λ = absorbance of a solution of unit concentration in a unit path length at wavelength λ.
It is a measure of the transition probability.

For mixtures absorbing at the same λ;

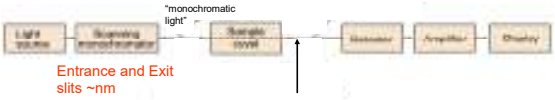
$A_\lambda = \Sigma\, \varepsilon_{\lambda,i} bc_i$



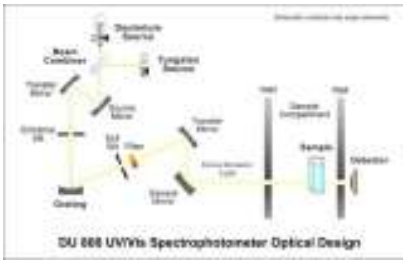
Single beam spectrometer:

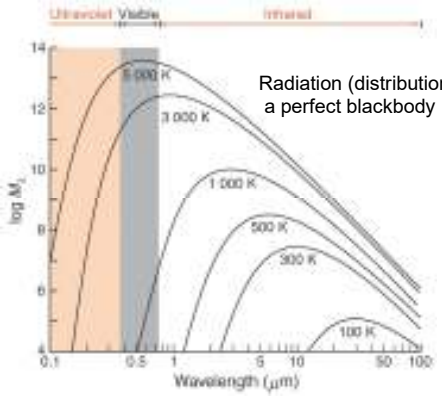
$$\log_{10}\frac{P_0}{P_{out}} = A = \varepsilon bc$$

Calibration P_0
Measurement P



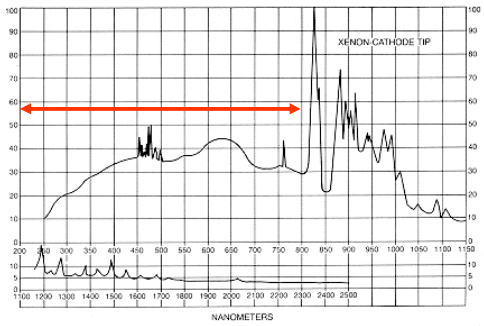
$P_{out} = P_0 10^{-\varepsilon bc}$ ← Note the form





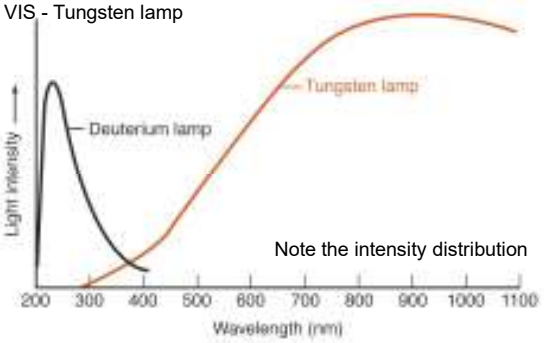
Radiation (distribution) from a perfect blackbody

Xenon lamp emission spectrum



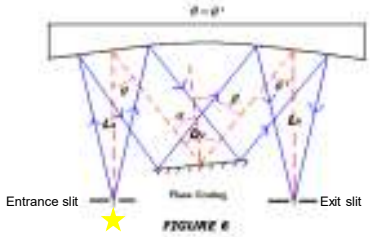
UV-VIS Sources:

UV - Deuterium lamp
VIS - Tungsten lamp

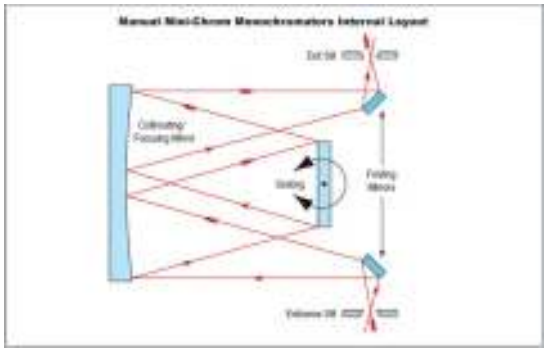


Note the intensity distribution

A monochromator configuration:

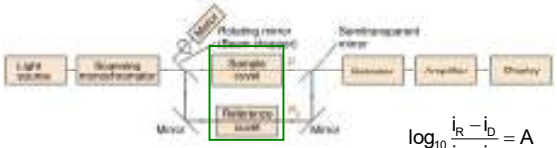


Fastie-Ebert Configuration



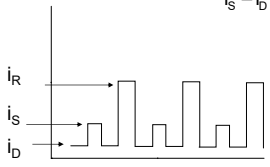
Double beam spectrometer:

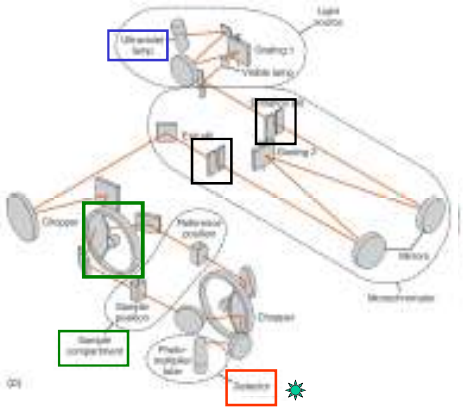
$$\log_{10}\frac{P_0}{P_{out}} = A = \varepsilon bc$$



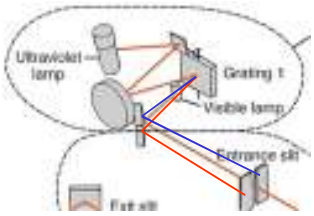
$$\log_{10}\frac{i_R - i_D}{i_S - i_D} = A$$

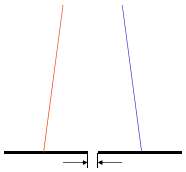
DOUBLE-BEAM
to correct for noise,
drift and other Instabilities.





Non-linearity of calibration curve: Instrumentation effect
Slit width



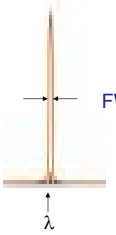


Band width ~ physical slit width

A range of wave lengths (more than one, for sure) enters the sample - polychromatic beam.

Monochromator Band Width

Rays exiting the monochromator is strictly *not* monochromatic. Only *nearly monochromatic*, has a wavelength distribution around (band width) λ, commonly referred to as monochromatic radiation wavelength. The smaller the band width (FWHM) of the beam, the better is the system.



Non monochromatic radiation leads calibration plots to deviate from linearity. Illustration:

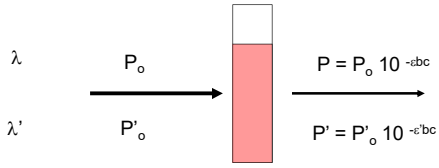
For a 'monochromatic' beam λ,

$\log (P_o/P) = A = \varepsilon bc = kc$ (11)

$P_o/P = 10^{\varepsilon bc}$ (12)

Similarly, at λ', $P_o/P' = 10^{\varepsilon' bc}$ (13)

The radiant power of two wavelengths passing through the solvent is given by $P_o + P'_o$, and that passing through the solution containing absorbing species by $P + P'$.



The combined absorbance is

$A_c = \log [(P_o + P'_o)/(P + P')]$ (14)

Substituting for P and P', we obtain;

$A_c = \log [(P_o + P'_o)/(P_o\ 10^{-\varepsilon bc} + P'_o 10^{-\varepsilon' bc})]$ (15)

Non-linearity arises *because molar absorptivity is different for the two wavelengths; seen clearly at high c values*.

$P_{out} = (P_0 10^{-\varepsilon bc} + P_0' 10^{-\varepsilon' bc})$

$P_{in} = (P_0 + P_0')$

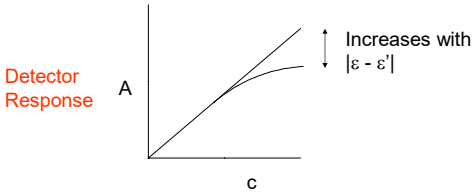
$P_{out} \neq P_{in} 10^{-kbc}$ not in the Beer's Law form $P_{out} = P_{in} 10^{-\varepsilon bc}$

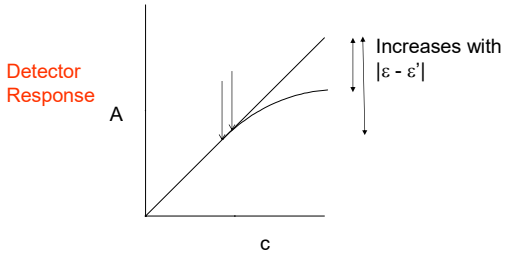
The combined absorbance is

$A_c = \log [(P_o + P'_o)/(P + P')]$ (14)

Substituting for P and P', we obtain;
$A_c = \log [(P_o + P'_o)/(P_o\, 10^{-\varepsilon bc} + P'_o 10^{-\varepsilon' bc})]$ (15)

Non-linearity arises *because molar absorptivity is different for the two wavelengths*.





Substituting for P and P', we obtain;
$A_c = \log [(P_o + P'_o)/(P_o\, 10^{-\varepsilon bc} + P'_o 10^{-\varepsilon' bc})]$ (15)

If *molar absorptivity is the same for the two wavelengths;*

$$P_{out} = (P_0 10^{-\varepsilon bc} + P_0^{'} 10^{-\varepsilon' bc})$$

$$P_{in} = (P_0 + P_0^{'}) 10^{-\varepsilon bc} \quad \text{(if } \varepsilon = \varepsilon' \text{)}$$

$$P_{out} = P_{in} 10^{-\varepsilon bc} \quad \text{the Beer's Law form !!}$$

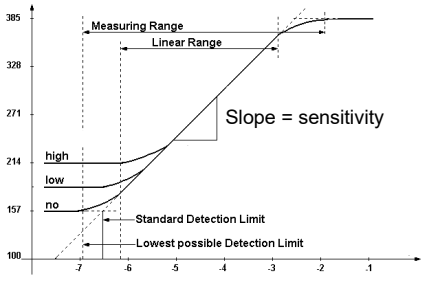
Detector Response

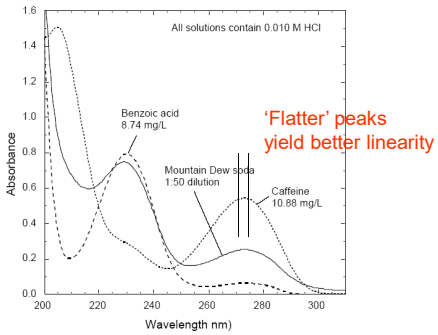
The dynamic range of a detector is that concentration range over which a concentration dependent output is produced.

The minimum of the range will be the concentration at which the output is at the detection limit and the maximum that concentration where the detector no longer responds to a concentration increase.

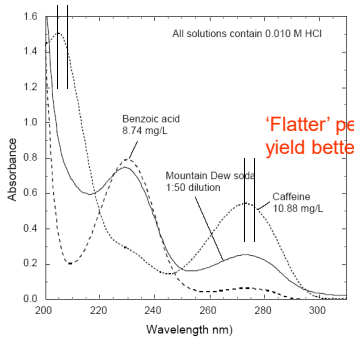
The dynamic range is usually given as a concentration ratio and is thus, dimensionless.

The linear dynamic range of a detector is that concentration range over which the detector output is linearly related to solute concentration.



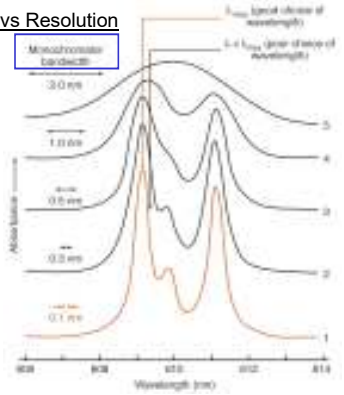


'Flatter' peaks yield better linearity



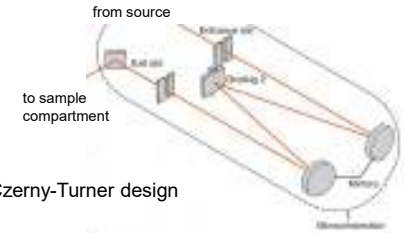
'Flatter' peaks yield better linearity

Bandwidth vs Resolution



Resolution: ~ Ability to distinguish consecutive peaks.

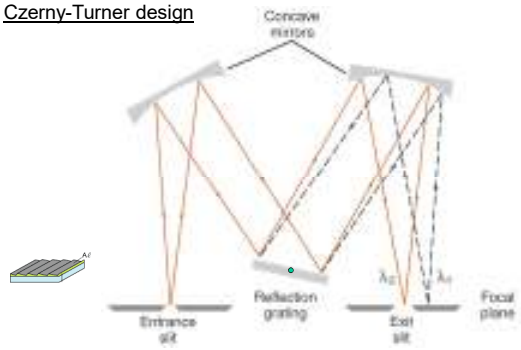
Typical Optical Path: Monochromator



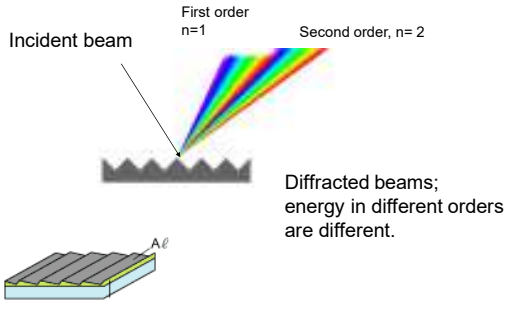
Czerny-Turner design

Monochromator bandwidth is directly related to the slit widths in addition to grating characteristics.

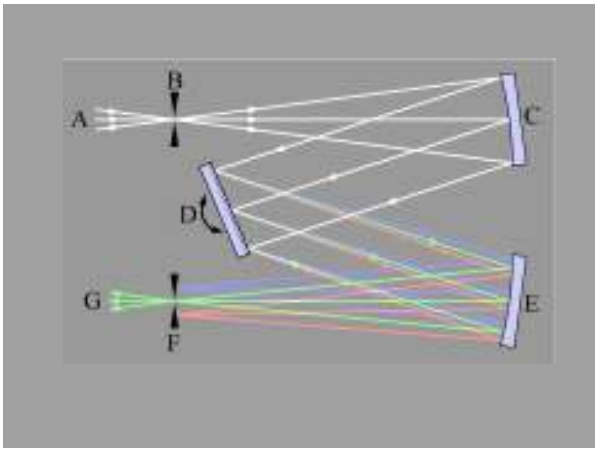
Czerny-Turner design

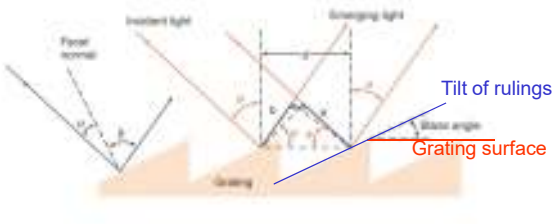


Turning the grating around it's center allows "different monochromatic λ" s to leave the exit slit of monochromator.



Diffracted beams; energy in different orders are different.

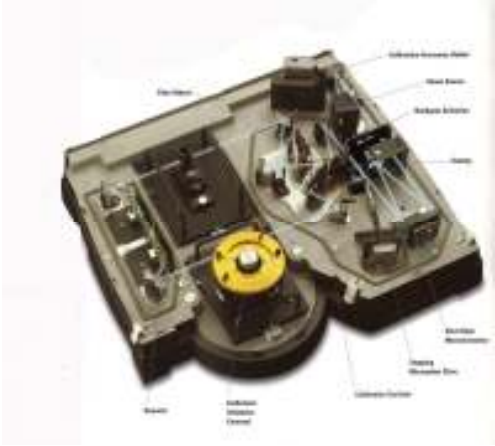




$$d = \frac{1}{\#\text{lines per unit length}}$$

Energy maximum for λ diffracted such that α=β.

http://www.shimadzu.com/products/opt/oh80jt0000001uz0.html



Angular Dispersion of Gratings

Dispersion is a 'measure' of the angle the grating must be rotated to change wavelength of the the exiting light by a unit wavelength.

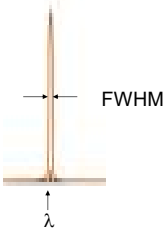
i.e. angular separation dϕ obtained for two wavelengths separated by dλ.

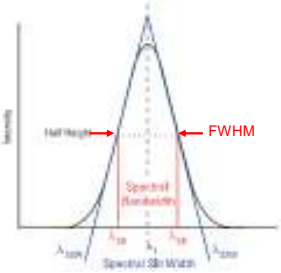
$$\frac{\Delta\phi}{\Delta\lambda} = \frac{n}{d\cos\phi}$$

Smaller d values of the grating generate larger dispersions leading to higher resolutions.

Monochromator Band Width

Rays exiting the monochromator is strictly not monochromatic. Only *nearly monochromatic*, has a wavelength distribution around (band width) λ, commonly referred to as monochromatic radiation wavelength. The smaller the band width (FWHM) of the beam, the better is the system.

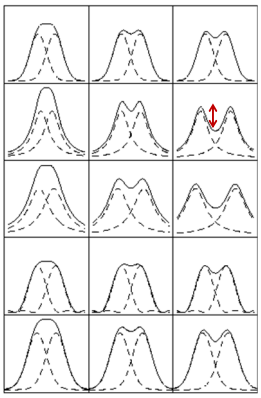




When a monochromator is set to a particular wavelength, light with a Gaussian intensity distribution of wavelengths emerges from the exit slit. The heavy black line represents the distribution of light that reaches the sample. The spectral bandwidth is defined as the FWHM.

Spectral slit width is defined as the total spread of wavelengths represented by the blue lines.

The spectral bandwidth will always be narrower than the spectral slit width.



Two consecutive peaks with Δλ having a valley of 5% are said to be resolved peaks.

$$R = \frac{\lambda}{\Delta\lambda} = nN$$

Resolution

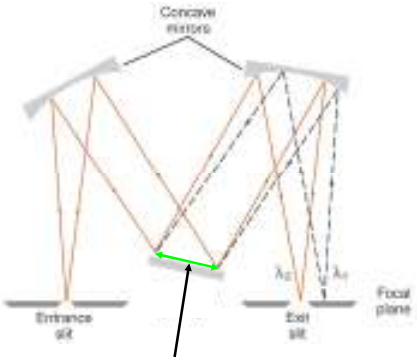
Resolution is defined as the ability of an instrument to separate light into finite, distinct wavelength regions and distinguish these finite regions from each other. It is primarily governed by the physical slit width of the instrument.

$$R = \frac{\lambda}{\Delta\lambda} = nN$$

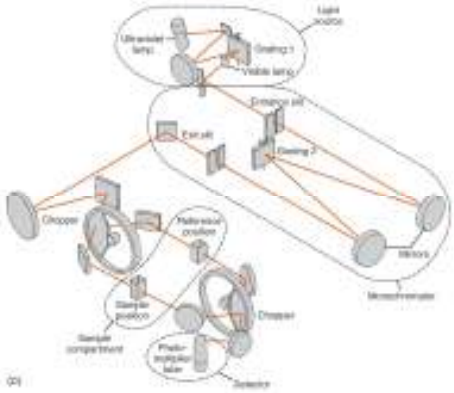
n = order
N= # grooves illuminated

The above in combination with the inherent angular dispersion of light from the exit of the monochromator to the detector of the instrument determines resolution.

Spectral resolution is a measure of the ability of an instrument to differentiate between two adjacent wavelengths λ and $\lambda+\Delta\lambda$.

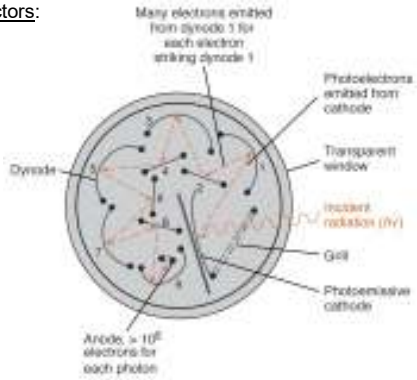


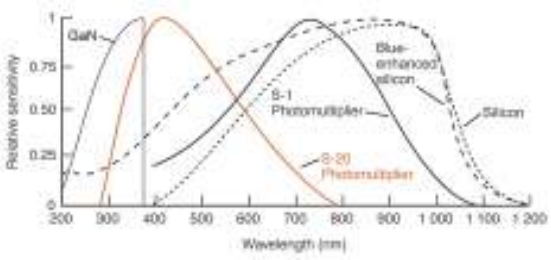
Illuminated region



Detectors:

PMT





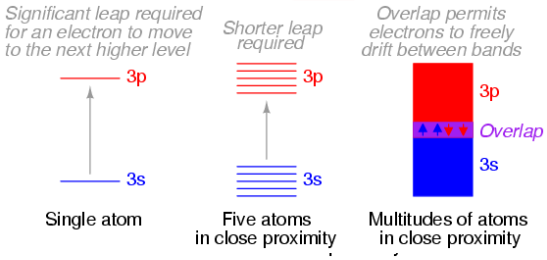
Note: uneven response

Detectors:
Diode Array Detector

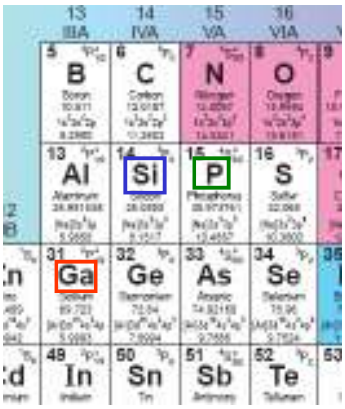
Diodes (semiconductor)

-Si-Si-Si-Si-Si-Si-
| | | | | |
-Si-Si-Si-Si-Si-Si-
| | | | | |
Si-Si-Si-Si-Si-Si-
| | | | | |
Si-Si-Si-Si-Si-Si-

Si Crystal

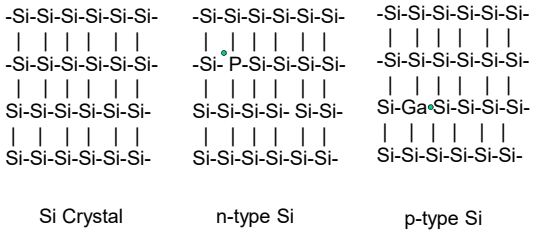


http://www.allaboutcircuits.com/vol_3/chpt_2/4.html



Detectors:

Diodes (semiconductor)

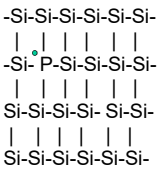


Si Crystal

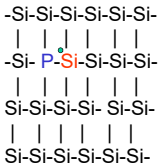
n-type Si

p-type Si

Detectors:

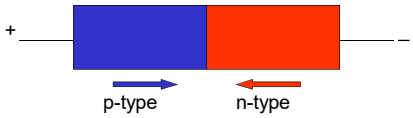


n-type Si

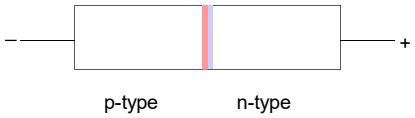


n-type Si

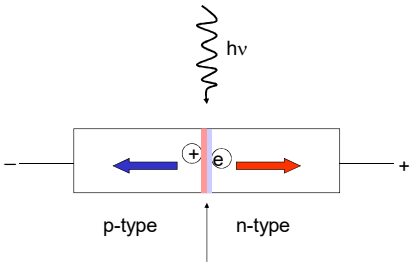
p-type n-type



Foward bias
$i \neq 0$



Reverse bias
$i = 0$

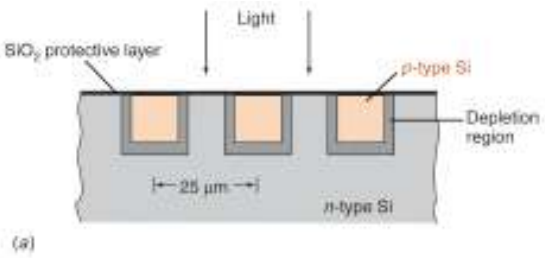


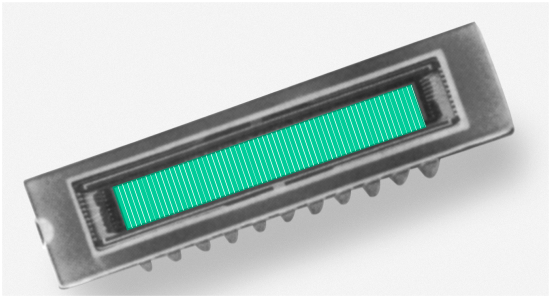
Reverse bias

Current (signal) produced is proportional number of photons falling (i.e. intensity of light) at the junction.

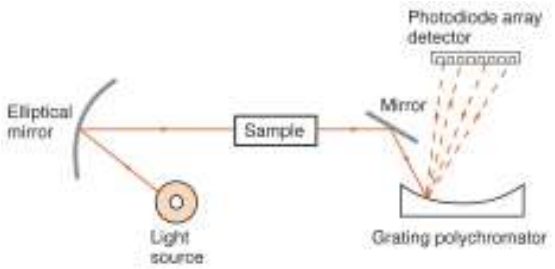
Detectors:

Diode Array

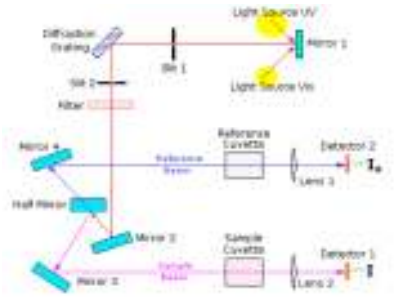




Diode-Array Spectrophotometer



Double Beam Spectrophotometer



Optical system of a double-beam spectrophotometer

UV-VIS in Quantitative Analysis

Inherently UV-VIS absorbing species
Organic chromophores, transition metal ions oxoanions, elemental halogens.

Non-absorbing species may be converted to absorbing species quantitatively.

$Fe^{+3} + SCN^{-} \rightarrow FeSCN^{+2}$
$Cr^{+3} \rightarrow CrO_4^{-2}$
$Ni^{+2} \rightarrow$ Ni-DMG
$Pb^{+2} \rightarrow$ Pb-Diphenyl-thiocarbazone

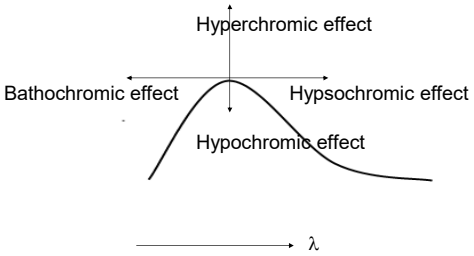
General Procedure (Single species)

a. select a λ most sensitive/less susceptible to slight drifts in λ (better reproducibility).
b. choose the solvent (medium) to disperse the 'test' and standards.
solvent, pH, temperature, ionic strength and interferents.
c. matched cells (clean, scratch less, dust free)
d. generate a *calibration curve* (A vs. c) : A = mc + b

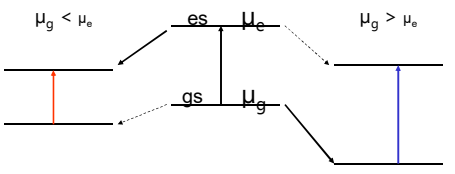
If media of 'tests' and standards are not comparable it is best to use standard addition method, not the *calibration curve* for quantitation.

Solvent Effects:

The color exhibited by a solute and/or the intensity of the peaks may vary with the nature of the solvent in which the chromophore is dissolved in.



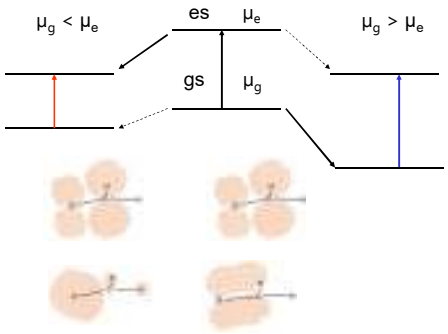
Solvent Effects:



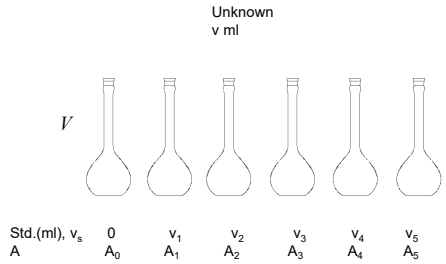
Polar solvent | Gas phase/ non-polar solvent | Polar solvent

Note: In general much more than the polarity of the solvent molecules is involved in solvent effects (solvatochromism) leading to wavelength shifts.

Solvent Effects:



Standard Addition Method

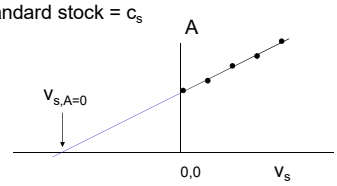


Volume of unknown = v
Concentration of unknown = c_u
Volume of standard addition = v_s
Concentration of the standard stock = c_s
Final volume = V

$c = (vc_u + v_s c_s)/V$

Using Beer's Law,

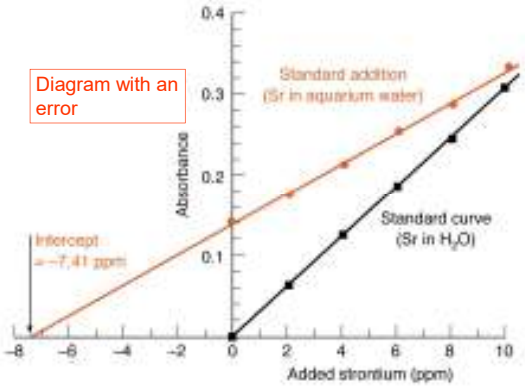
$A = k\dfrac{(vc_u + v_s c_s)}{V}$



Upon rearrangement; $\dfrac{AV}{k} = vc_u + v_s c_s$ where $k = \varepsilon l$

$0 = vc_u + v_{s,A=0} c_s$; ∵ at the x intercept, A=0

$\Rightarrow c_u = -\dfrac{v_{s,A=0} c_s}{v}$



Standard Addition Method (one addition):

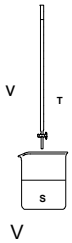
$A_0 = kvc_u / V$

$A_1 = k(vc_u + v_s c_s)/V$

$\dfrac{A_0}{A_1} = \dfrac{vc_u}{vc_u + v_s c_s}$

Solve for c_u.

Spectrometric/Photometric Titrations



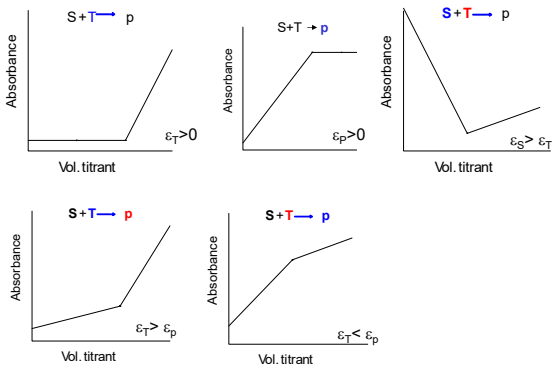
Analyte (S) + Titrant (T) → Products (p)

If one or more of T, S and p colored, spectrophotometric titrations possible.

Sample reaction mixture after each addition of each aliquot (v), measure A_{obs}.

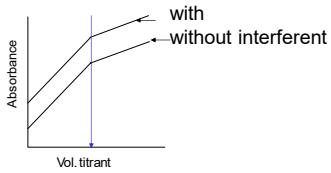
Corrected Absorbance

$A = A_{obs}\dfrac{V + v}{V}$

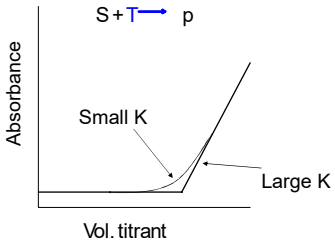


Advantages of Spectrophotometric Titration:

1. Calculations based on multiple measurements, minimizes errors.
2. Change of Absorbance is monitored, interferents do not contribute to the final calculation..



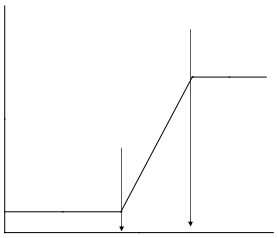
3. Extrapolation: to determine equivalence point. Reactions with relatively low K can be analyzed.



4. Successive titrations possible. e.g. Bi^{+2}, Cu^{+2}, vs EDTA.

$K_{Bi\text{-}EDTA} > K_{Cu\text{-}EDTA}$

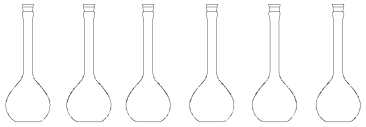
@745nm



Standard Addition Method (modification)

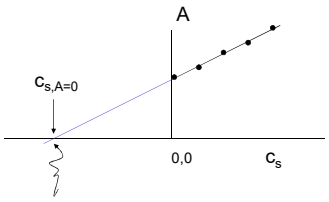
Series of standards prepared
Mix standards with unknown; Unknown volume = volume of standard = v ml

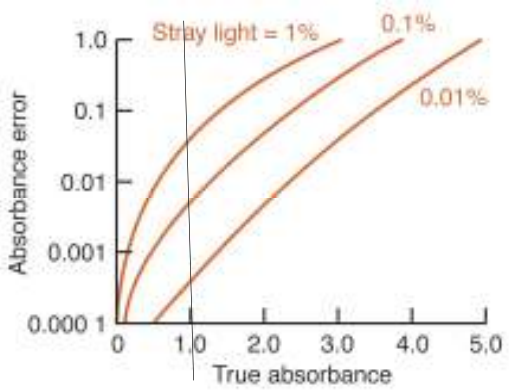
$V = 2v$



Conc. Std C_s, V	0	c_1	c_2	c_3	c_4	c_5
A	A_0	A_1	A_2	A_3	A_4	A_5

$$c = (vc_u + vc_s)/V$$
$$A = k(vc_u + vc_s)/V$$
$$AV = kvc_u + kvc_s$$
$$A \times 2v = kvc_u + kvc_s$$
$$0 = kc_u + kc_{s,A=0}$$
$$c_u = -c_{s,A=0}$$





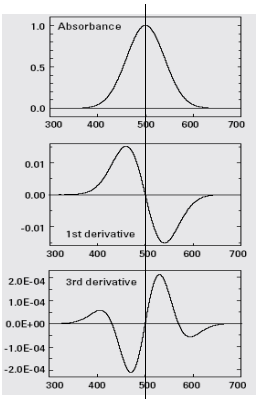
Derivative Spectroscopy

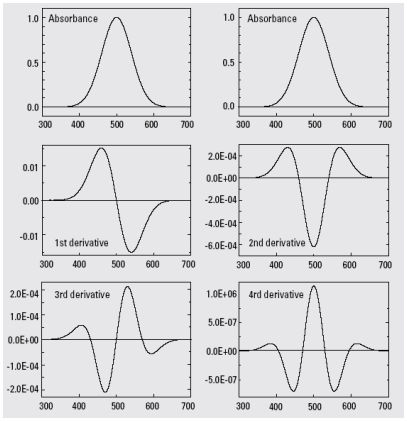
First or higher derivatives of absorbance with respect to wavelength for qualitative analysis and for quantification.

$A = f(\lambda)$ zeroth order

$\frac{dA}{d\lambda} = f'(\lambda)$ first order

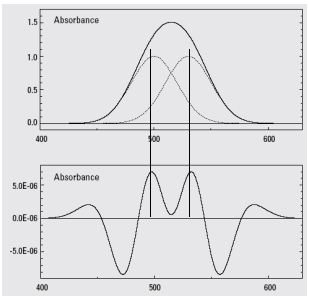
$\frac{d^2A}{d\lambda^2} = f''(\lambda)$ second order

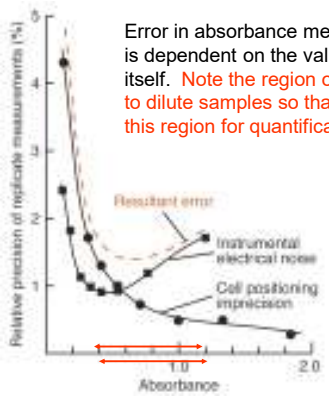




Resolution enhancement

qualitative analysis: to identify the presence of two analytes with Very similar λ_{max} values that are not resolved in the absorbance spectrum.





Error in absorbance measurements is dependent on the value of the absorbance itself. Note the region of least error. Advisable to dilute samples so that absorbance falls in this region for quantification..

Jobs Method: Empirical Formula of binary compounds/complexes using the *method of continuous variation* or Jobs Method.

$$P + X = P_nX_m$$

The amount of one of the components is varied from a small value to a large value while the amount of the other component is varied from large to small. The amount of the product formed is monitored via the absorbance of the product.

$aP + bX = dD$

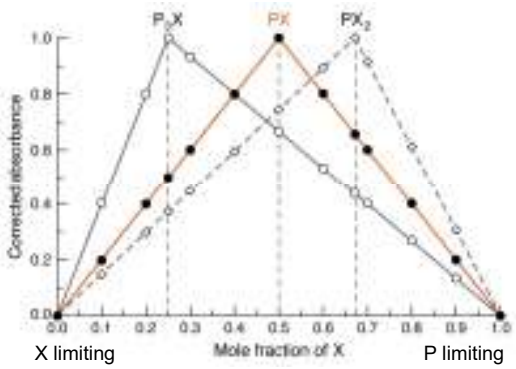
$P + kX = mD$ $k=b/a$ $m= d/a$

If a series of solutions is made, each containing the ***same total number of moles*** of P and X, ***but a different ratio,*** R = moles P/moles X, the maximum amount of product, D, is obtained in the solution in which R = k (the stoichiometric ratio).

When R > k, there is an excess of reagent X, so reagent P is the limiting reagent and X is in excess.

when R < k, there is an excess of reagent P, and X is limiting reagent and P is the limiting reagent.

$aP + bX = dD = P_aX_b$



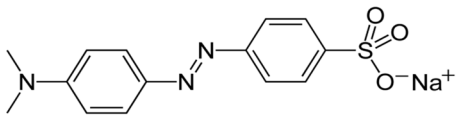
Isobestic Point:

A ↔ B (Both absorb, generate spectra.)
A = B + H^+

This term is usually employed with reference to a set of absorption spectra, plotted on the same chart for a set of solutions in which the sum of the concentrations of two principal absorbing components, A and B, is constant.

The curves of absorbance against wavelength (or frequency) for such a set of mixtures often all intersect at one or more points, called isobestic points.

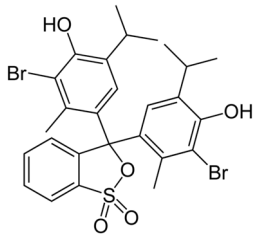
Methyl Orange



Acidic to alkaline solutions (left to right).

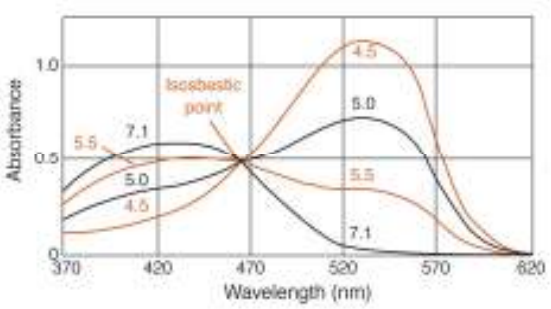
http://en.wikipedia.org/wiki/Methyl_orange

Bromothymol-blue



acidic, neutral, and alkaline solutions (left to right).

http://en.wikipedia.org/wiki/Bromothymol_blue



Molar absorptivity of A and B same at isobestic point.

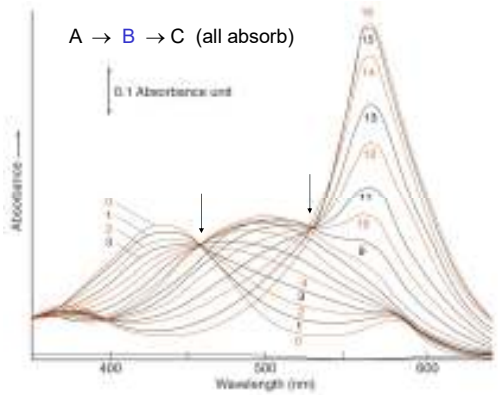
Isosbestic points are commonly met when electronic spectra are taken;

(a) on a solution in which a chemical reaction is in progress (in which case the two absorbing components concerned are a 'reactant' and a 'product'),
or (b) on a solution in which the two absorbing components are in equilibrium and their relative proportions are controlled by the concentration of some other component,

$$A \rightleftharpoons B + H^+$$

.

In all these examples, A (and/or B) may be coming from a single *chemical species* or a mixture of chemical species present in invariant proportion.



Evidence of an intermediate of a reaction.

If absorption spectra of the types considered above intersect at many isosbestic points, this is *prima facie evidence* in case;

(a) for the formation of a *reaction intermediate* in substantial concentration,

$$A \rightarrow B \rightarrow C$$

(b) for the involvement of a third absorbing species in the equilibrium, e.g.

$$A \rightleftharpoons B + H^+ \rightleftharpoons C + 2H^+$$