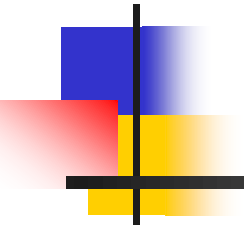
Biofyzikální chemie II

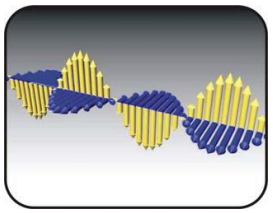
„ Student není pohár, který můžeme naplnit, student je pochodeň, kterou můžeme zapálit.“



UV-Vis spectra

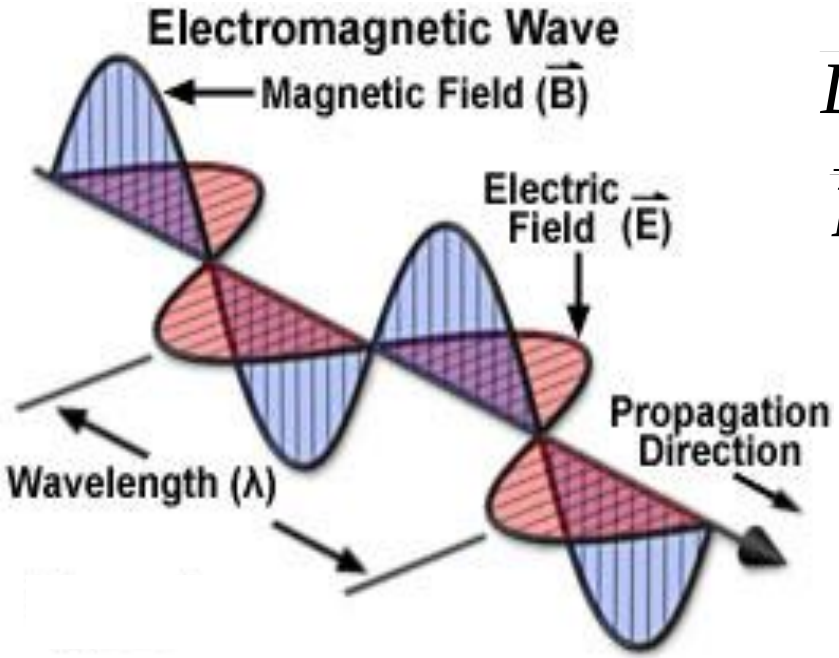
1. Basic principles
2. QM of interaction between light and molecules
3. Absorption spectroscopy of electronic states
4. Instrumentation - spectrophotometry
5. Spectroscopic analysis of biopolymers
6. Effect of conformation on absorption

What is light?



According to Maxwell, light is an electromagnetic field characterized by a frequency *ν*, velocity c, and wavelength λ. Light obeys the relationships:

James Clerk **Maxwell** (1831-1879)



$$\vec{D} = \varepsilon \vec{E}$$

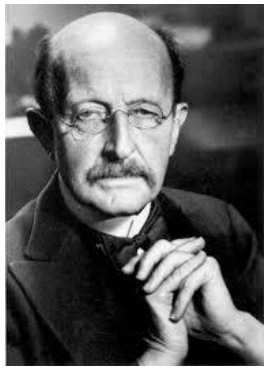
$$\vec{B} = \mu \vec{H}$$

$$\frac{1}{\sqrt{\varepsilon_0 \mu_0}} = c$$

$$\varepsilon_0 = 8.859.10^{-12}\ Fm^{-1}$$

$$\mu_0 = 4\pi.10^{-7}\ Hm^{-1}$$

Max Karl Ernst Ludwig **Planck** (1858-1947)



http://www.edumedia-sciences.com/en/a185-transverse-electromagnetic-wave

$$E = h\nu = h\frac{c}{\lambda}$$

$$\nu = \frac{c}{\lambda} \qquad \tilde{\nu} = \frac{1}{\lambda} \qquad \nu = \tilde{\nu}.c$$

h…….Planck constant 6.63 .10^{-34} J.s

Maxwell equations

Name	Integral equations	Differential equations
Gauss's law	$\oiint_{\partial\Omega} \mathbf{E}\cdot\mathrm{d}\mathbf{S} = \frac{1}{\varepsilon_0}\iiint_{\Omega}\rho\,\mathrm{d}V$	$\nabla\cdot\mathbf{E} = \frac{\rho}{\varepsilon_0}$
Gauss's law for magnetism	$\oiint_{\partial\Omega} \mathbf{B}\cdot\mathrm{d}\mathbf{S} = 0$	$\nabla\cdot\mathbf{B} = 0$
Maxwell–Faraday equation (Faraday's law of induction)	$\oint_{\partial\Sigma} \mathbf{E}\cdot\mathrm{d}\boldsymbol{\ell} = -\frac{d}{dt}\iint_{\Sigma}\mathbf{B}\cdot\mathrm{d}\mathbf{S}$	$\nabla\times\mathbf{E} = -\frac{\partial\mathbf{B}}{\partial t}$
Ampère's circuital law (with Maxwell's addition)	$\oint_{\partial\Sigma} \mathbf{B}\cdot\mathrm{d}\boldsymbol{\ell} = \mu_0\iint_{\Sigma}\mathbf{J}\cdot\mathrm{d}\mathbf{S} + \mu_0\varepsilon_0\frac{d}{dt}\iint_{\Sigma}\mathbf{E}\cdot\mathrm{d}\mathbf{S}$	$\nabla\times\mathbf{B} = \mu_0\left(\mathbf{J} + \varepsilon_0\frac{\partial\mathbf{E}}{\partial t}\right)$

- $\int$ denotes an integral,
- Ω denotes a volume, and $\partial\Omega$ is the closed surface enclosing it, with normal directed outwards
- $\mathrm{d}V$ denotes a differential volume element of Ω,
- Σ denotes a non-closed surface (assumed to be time independent),
- $\mathrm{d}\mathbf{S}$ denotes a differential vector area element of $\partial\Omega$ or Σ, parallel to the surface normal, and
- $\partial\Sigma$ is the closed loop circulating around Σ, counterclockwise (in accordance to $\mathrm{d}\mathbf{S}$).

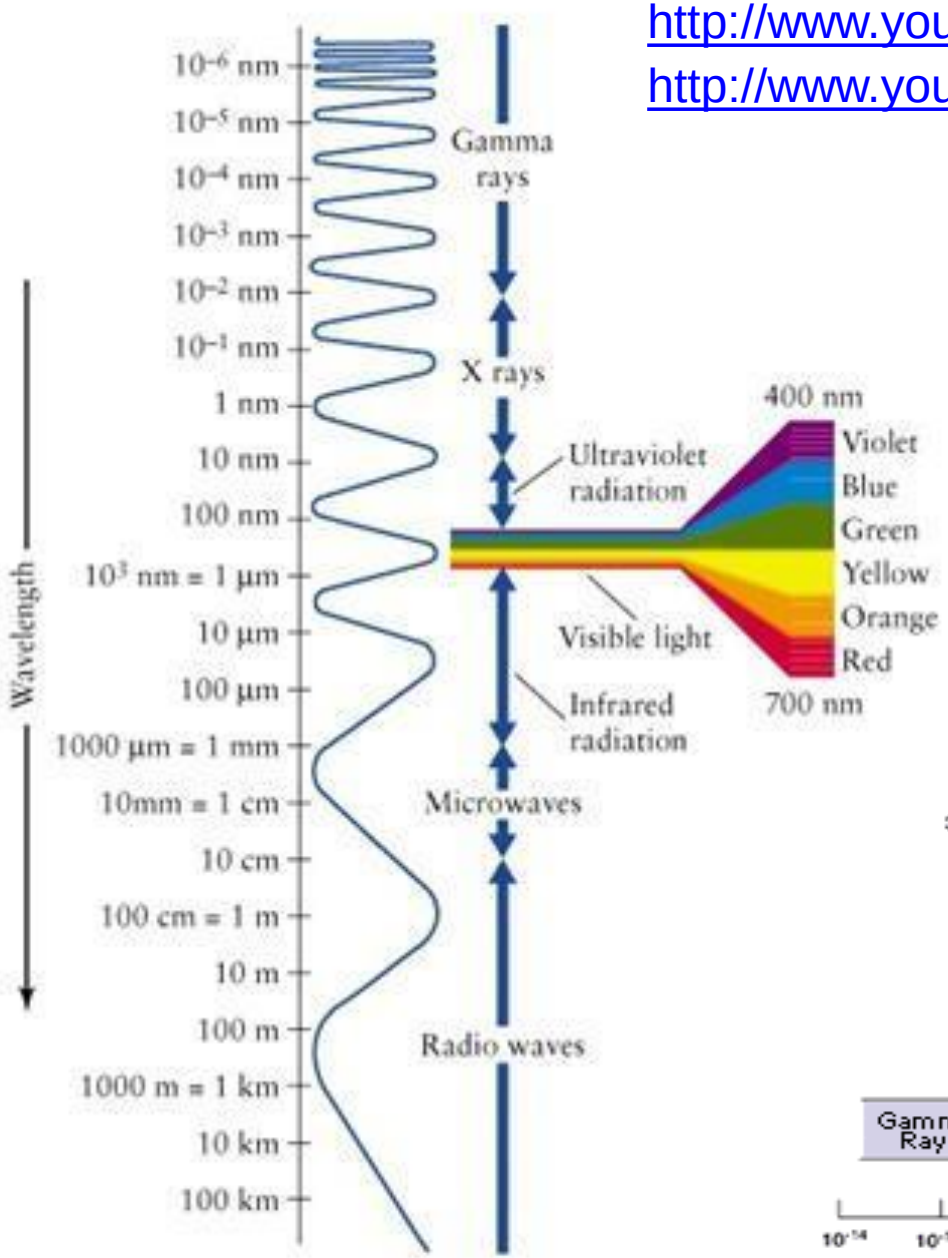
- ∇ is the Del operator
- $\mathbf{E}$ is the electric field,
- $\mathbf{B}$ is the magnetic field,
- $\mathbf{J}$ is the total current density,
- ρ is the total charge density,
- ε_0 is the permittivity of free space,
- μ_0 is the permeability of free space

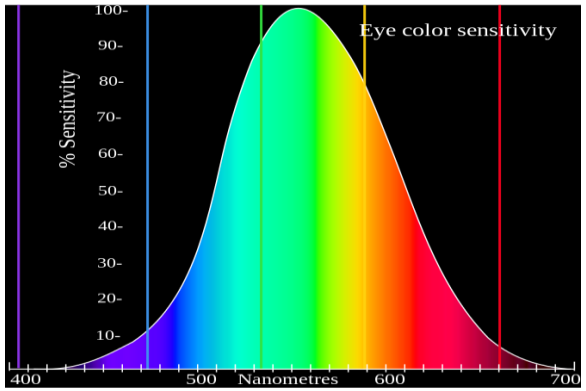
Maxwell's equations describe how electric and magnetic fields are generated and altered by each other and by charges and currents. They are the foundation of classical electrodynamics, classical optics, and electric circuits.

The electromagnetic spectrum

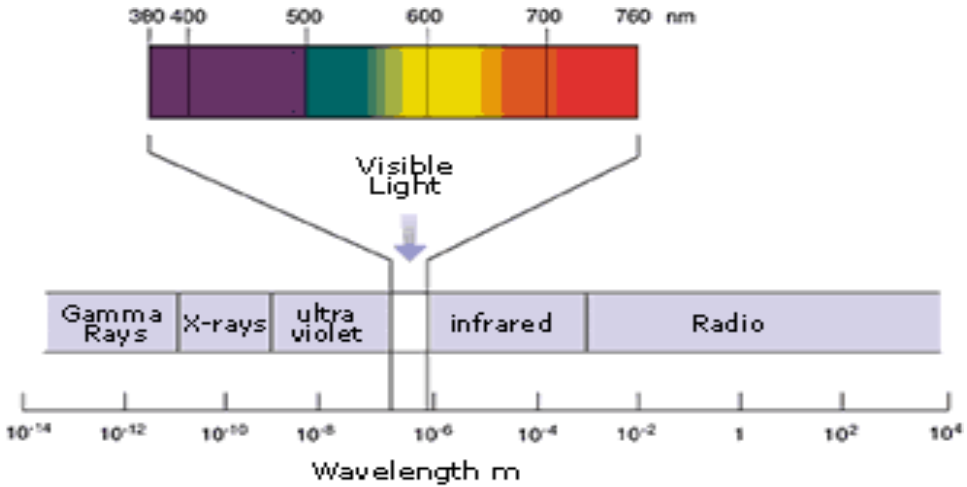
http://www.youtube.com/watch?v=cfXzwh3KadE

http://www.youtube.com/watch?v=m4t7gTmBK3g





Relative brightness sensitivity of the human visual system as a function of wavelength



Biologically useful spectroscopic regions in UV-Vis-IR

Wavelength (cm^{-1})	Energy (aprox.) (J/mol)	Spectroscopic region	Techniques /Applications
~ 10^{-5}	1250	vacuum UV	electronic spectra
3.10^{-5}	420	near UV	electronic spectra
carbon-carbon bond energy			
6.10^{-5}	200	visible	electronic spectra
~ 10^{-3}	15	IR	vibrational spectra
RT at ambient temperature			
10^{-2}	1.5	Far IR	vibrational spectra

There is no simple way to explain the interaction of light with matter. Why?

Light is rapidly oscillating electromagnetic field. Molecules contain distribution of charges and spin that have electrical and magnetic properties and these distributions are altered when a molecule is exposed to light.

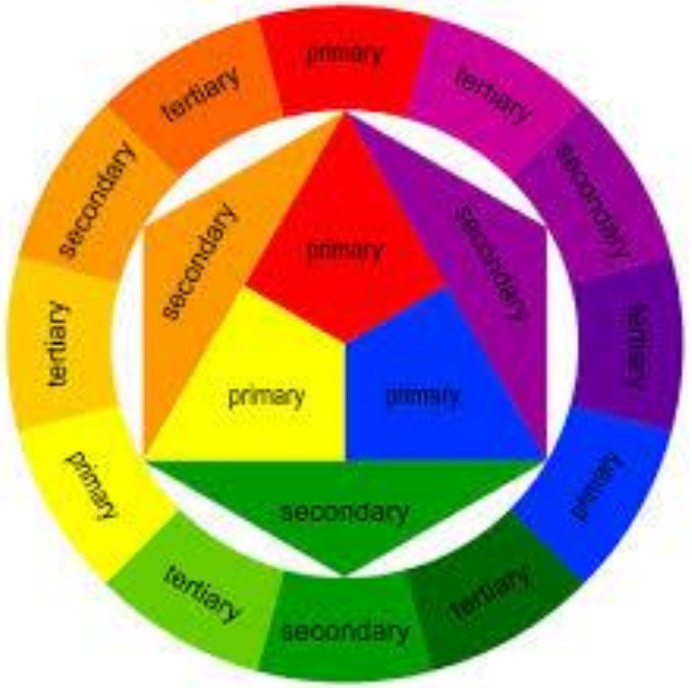
Explanation:

1. The rate at which the molecule responds to this perturbation
2. Why only certain wavelength cause changes in the state of the molecule
3. How the molecule alters the radiation

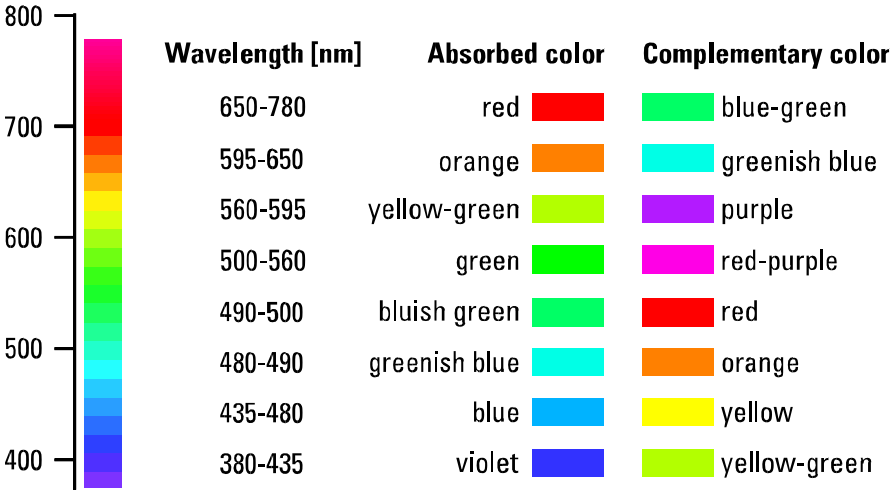
Transmission and color

color	wavelength interval	frequency interval
red	~ 700–635 nm	~ 430–480 THz
orange	~ 635–590 nm	~ 480–510 THz
yellow	~ 590–560 nm	~ 510–540 THz
green	~ 560–490 nm	~ 540–610 THz
blue	~ 490–450 nm	~ 610–670 THz
violet	~ 450–400 nm	~ 670–750 THz

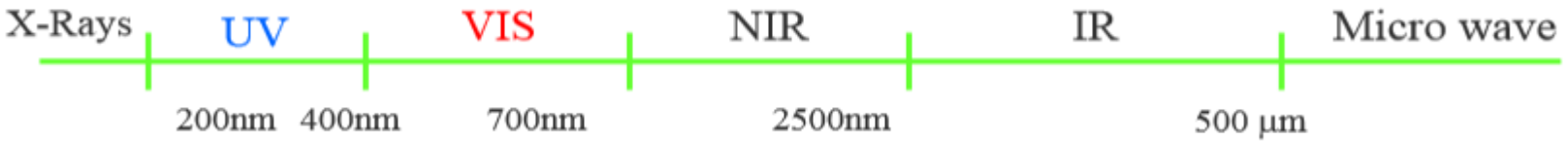
Oswald circle



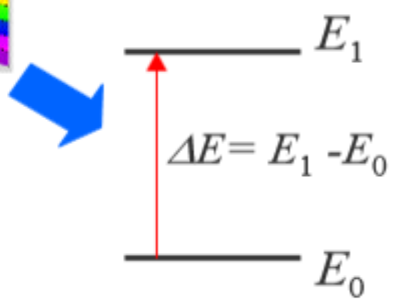
The human eye sees the complementary color to that which is absorbed



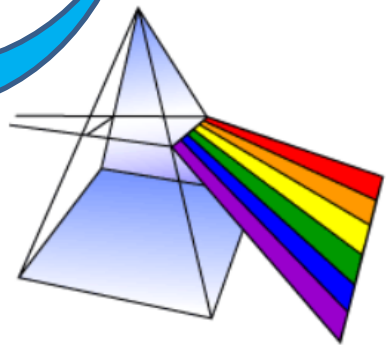
Type of transition

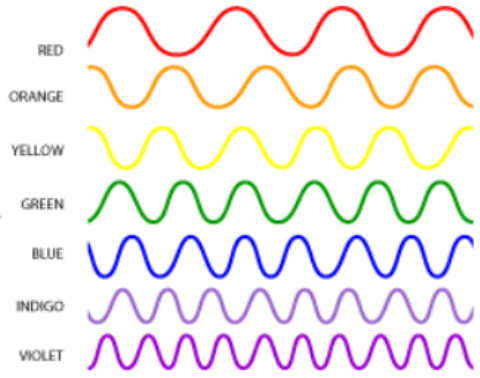


Type of Radiation	Type of Transition
X-Rays	Inner Electron
UV/VIS	Outer Electron
IR	Molecular vibration
Micro Wave	Molecular rotation



(1) π, σ, and n electrons
(2) *d* and *f* electrons
(3) charge transfer electrons





Each color in a rainbow corresponds to a different wavelength of electromagnetic spectrum.

INTERACTION BETWEEN LIGHT AND MOLECULES

Calculation of properties of molecules by QM

1. The state of a system is described by a wavefunction
2. An observable quantity (E, μ, the location in space) is governed by a mathematical device known as an operator
3. The result of a measurement on a state can be computed by taking the average value of operator on that state
4. A transition between two state can be induced by a perturbation which is measured by an operator (deform the initial state - resembling final state)
5. The ability of light to induce transitions in molecules can be calculated according to its ability to induce μ(s) that oscillate with the light.
6. The preferred directions for inducing dipole moments μ(s) are fixed with respect to the geometry of the molecule.

Ad 1. The state of a system is described by a wavefunction (ψ)

is not a directly measurable quantity and it is often referred to as a probability amplitude

$$P = \psi^* \psi \qquad \psi = a + bi \qquad \psi^* = a - bi \longrightarrow P = a^2 + b^2$$

$$\int P\, d\tau = \int \psi^* \psi\, d\tau = \langle \psi | \psi \rangle = 1$$

$\langle \psi | \psi \rangle$ Dirac notation

ψ_a, ψ_b normalized functions for two states $\langle \psi_a | \psi_a \rangle = 1$ $\langle \psi_b | \psi_b \rangle = 1$

the maximum value $\langle \psi_a | \psi_b \rangle = 1$ the minimum value $\langle \psi_a | \psi_b \rangle = 0$ the functions are ortogonal

$$\psi = C_a \psi_a + C_b \psi_b$$

$$\psi(r,s) = \psi(r)\sigma(s)$$

e electron, N nucleus

$$\psi = \psi_e(r,R)\phi_N(R)$$ Born-Opperheimer approximation

$$\psi = \psi_1(r_1) + \psi_2(r_2)$$ the state of two electrons without the interaction

$\hat{O}\psi = \Lambda\psi$ $\langle O \rangle = \int \psi^* \hat{O} \psi\, d\tau = \langle \psi | \hat{O} | \psi \rangle$ Λ is a pure number, the eigenvalue

The Schrödinger equation

time-independent (stationery state)

$$\hat{H}\psi = E\psi$$

time-dependent

$$i\hbar\, d\psi / dt = \hat{H}\psi$$

$\hat{H}$ Hamiltonian operator $\quad \langle \psi | \hat{H} | \psi \rangle = E \quad \hat{H} = \hat{T} + \hat{V}$

$\hat{T}$ the kinetic energy operator — $\hat{V}$ the potential energy operator

$$\sum_i \frac{1}{2} m_i v_i = \sum_i \frac{p_i^2}{2m_i}$$

$$\hat{T} = \sum_i \frac{\hat{p}_i^2}{2m_i} \quad where \quad \hat{p}_i^2 = -\hbar^2 \nabla^2$$

depends on the system

$$p_i^2 = -\hbar^2 \nabla^2$$

$$\psi(t) = \psi(0) e^{-iEt/\hbar}$$

$$P = \psi^*(t)\psi(t) = |\psi(0)|^2 e^{-iEt/\hbar} e^{+iEt/\hbar} = |\psi(0)|^2$$

ψ_1 .....E_1

ψ_2 .....E_2

$$\psi = C_1\psi_1 + C_2\psi_2$$

$$\psi = \sum_i C_i \psi_i$$

perturbation as a potential $\hat{V}$

ψ_a .....*original state*

ψ ..... *final state*

$$\langle \psi | \hat{V} | \psi_a \rangle = \sum_i C_i \langle \psi_i | \hat{V} | \psi_a \rangle$$

Interaction of light with molecules (chromophores)

For simplicity: I. What can be ignored?

1) … the magnetic vector, only the electric vector

2) … the spacial variation of the electric field of the light within the molecule

Chromophore ~10 Å  Wavelenght of light ~ 3 000 Å

The electric field felt by a molecule: $E(t) = E_0\, e^{i\omega t}$ $\left(\omega = 2\pi\nu = 2\pi\frac{c}{\lambda}\right)$

3) …. the effect of time?

ψ_a .....*original state*

ψ .....*final state*

What we need to compute?.... the rate at which light causes transitions between ψ_a and ψ

For simplicity: II. What can be ignored? …. other states

ψ_a .....*original state* ψ_b .....*final state*

Is the interaction (light – molecule) dependent on time?.... $\hat{H}' = \hat{H} + \hat{V}(t)$

$$\psi(t) = C_a(t)\,\psi_a e^{-iE_a t/\hbar} + C_b(t)\,\psi_b e^{-iE_b t/\hbar}$$

Interaction of light with molecules

$$\psi(t) = C_a(t)\,\psi_a e^{-iE_a t/\hbar} + C_b(t)\,\psi_b e^{-iE_b t/\hbar}$$

$$i\hbar\left(\psi_a(t)e^{-iE_a t/\hbar}dC_a/dt + \psi_b e^{-iE_b t/\hbar}dC_b/dt\right) = \hat{V}(t)\left[\psi_a e^{-iE_a t/\hbar}C_a(t) + \psi_b e^{-iE_b t/\hbar}C_b(t)\right]$$

C_a and C_b calculation: some approaches

1) … to expand the charge distribution in a multipole series ⟶ the electric dipole

e_i the electronic charge at the position $\hat{r}_i$
Born-Oppenheimer approximation

$$\hat{\mu} = \sum_i e_i \left|\hat{r}_i\right|$$

The interaction energy: $\hat{V}(t) = \hat{\mu} E_0 e^{i\omega t}$

$\hat{\mu}$ the dipole operator

- the spatial part from–x to + x, from–y to + y, from –z to + z
- E_o is constant
- $\langle\psi_a|\hat{\mu}|\psi_b\rangle = \langle\psi_b|\hat{\mu}|\psi_a\rangle$

$$h\nu = \hbar\omega = E_b - E_a$$

spectral bands - light absorption or light-induced transition
only at certain narrow wavelength (or frequency) interval

Interaction of light with molecules

!!! the rate at which molecules in stage ***a*** are transformed to state **b** by light = the rate of change of $|C_b(t)|^2$

$$\frac{dP_b}{dt} = \frac{d}{dt}\int |C_b(t)|^2 \, dv = \frac{1}{2\hbar^2} \left| \langle \psi_b | \hat{\mu} | \psi_a \rangle E_0 \right|^2$$

not only for *a* – *b* transition but also *b* – *a* transition

!!! the rate at which energy is taken up from the incident light beam

$$\frac{dP_b}{dt} = B_{ab} I(v)$$

B_{ab} is the transition rate per unit energy density of the radiation

$I(v)$ is the energy density incident on the sample at frequency *v* $I(v) = \frac{|E_0|^2}{4\pi}$

$$B_{ab} = (2/3)(\pi/\hbar^2) \left| \langle \psi_b | \hat{\mu} | \psi_a \rangle \right|^2$$

!!! the rate at which energy is removed from the light depends on the number of a-b absorption transitions stimulated by light

$$-\frac{dI(v)}{dt} = hv(N_a B_{ab} - N_b B_{ba}) I(v)$$

Einstein´s coefficients for stimulated absorption and emission

$B_{ab} = B_{ba}$ *simple cases*

Interaction of light with molecules

$$B_{ab} = (2/3)(\pi/\hbar^2)\left|\langle \psi_b | \hat{\mu} | \psi_a \rangle\right|^2$$

transition dipoles

$\langle \psi_b | \hat{\mu} | \psi_a \rangle$

light-induced dipole

$\mu_{ind} = \hat{\alpha} E$

the transition dipole moment - vector

MATRIX

B_{ab} - the transition probability

the ability of light to distort a molecule

not preferred direction, only a preferred orientation

from intensity $I(\nu)$ $\longrightarrow$ $\left|\langle \psi_b | \hat{\mu} | \psi_a \rangle\right|^2$ electronic distribution within the molecule

$I(\nu)$= f (the relative orientation of the molecule with) $\langle \psi_b | \hat{\mu} | \psi_a \rangle . E_0$

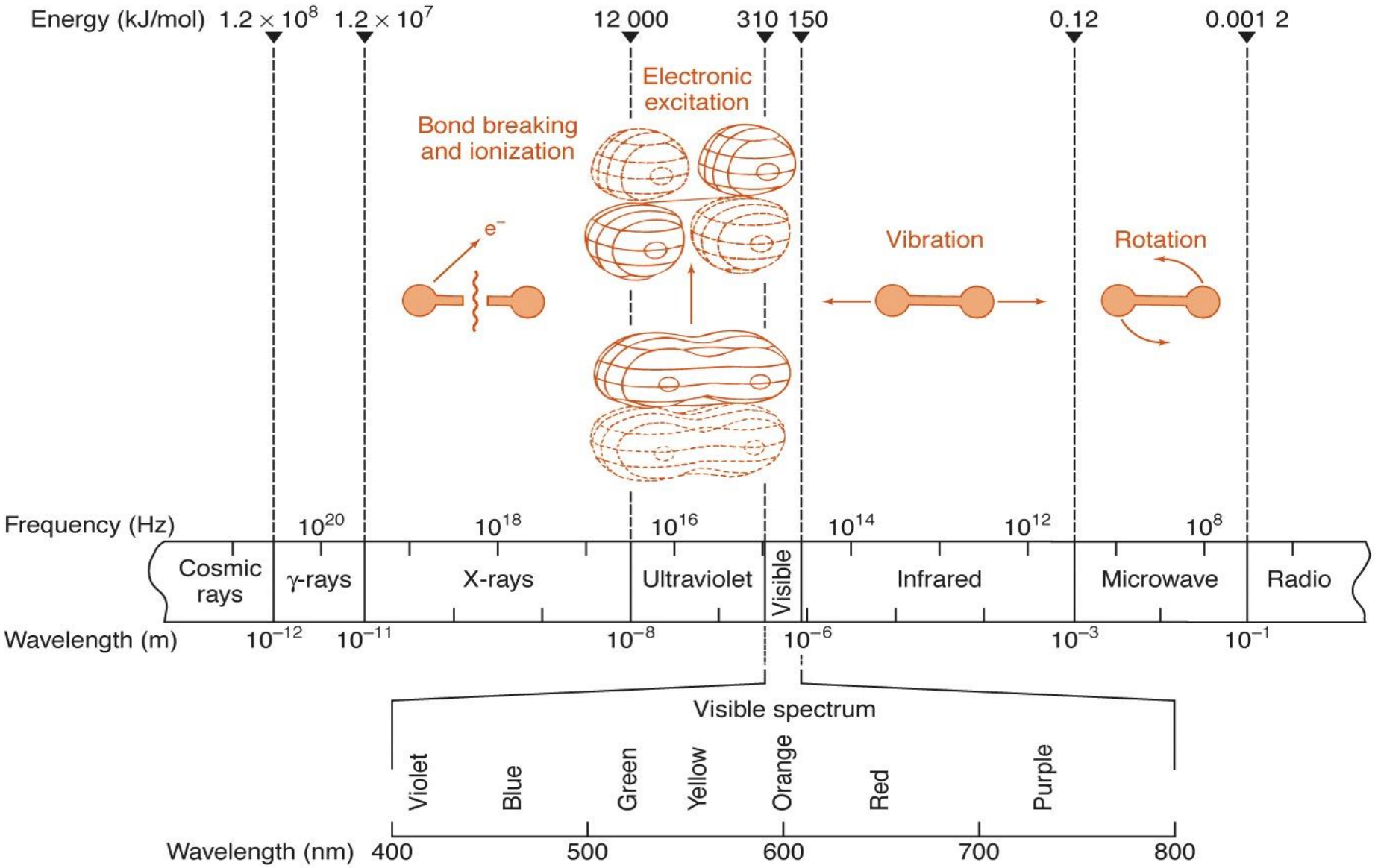
excitation in phase or out of phase

e.g. exempli gratia

	In phase	Out of phase by 180^0
Time = t	↑↑	↑↓
Time = t+1/2 ν	↓↓	↓↑

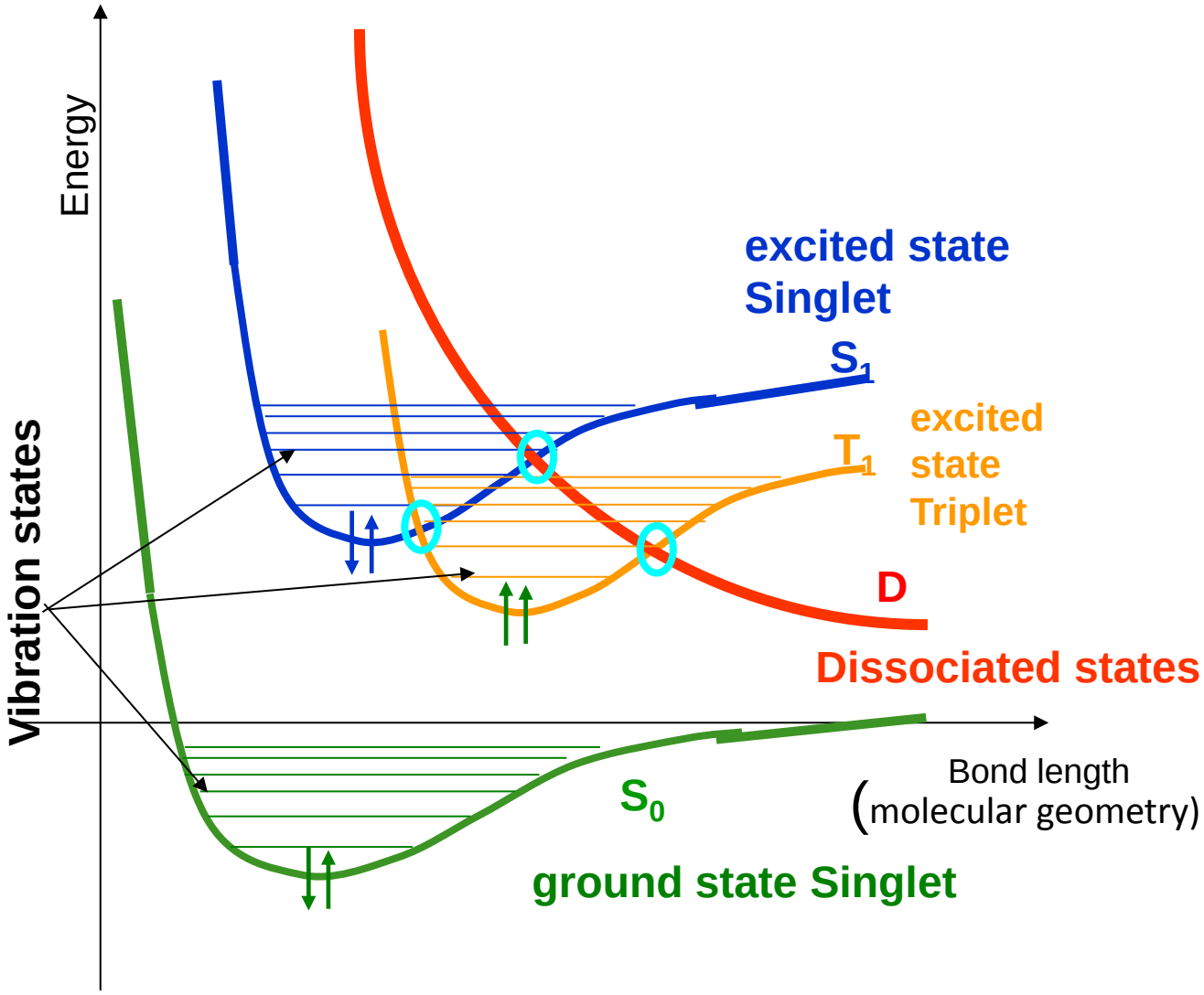
Induced neighboring chromophores – the interactions can be attractive or repulsive

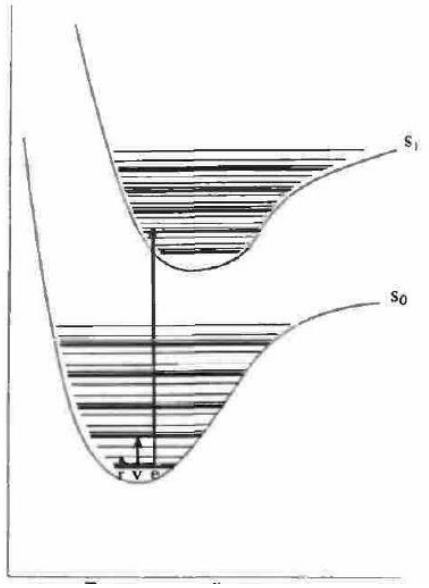
Regions of Electromagnetic Spectrum



ABSORPTION SPECTROSCOPY OF ELECTRONIC STATES

Electronic structures of simple molecule





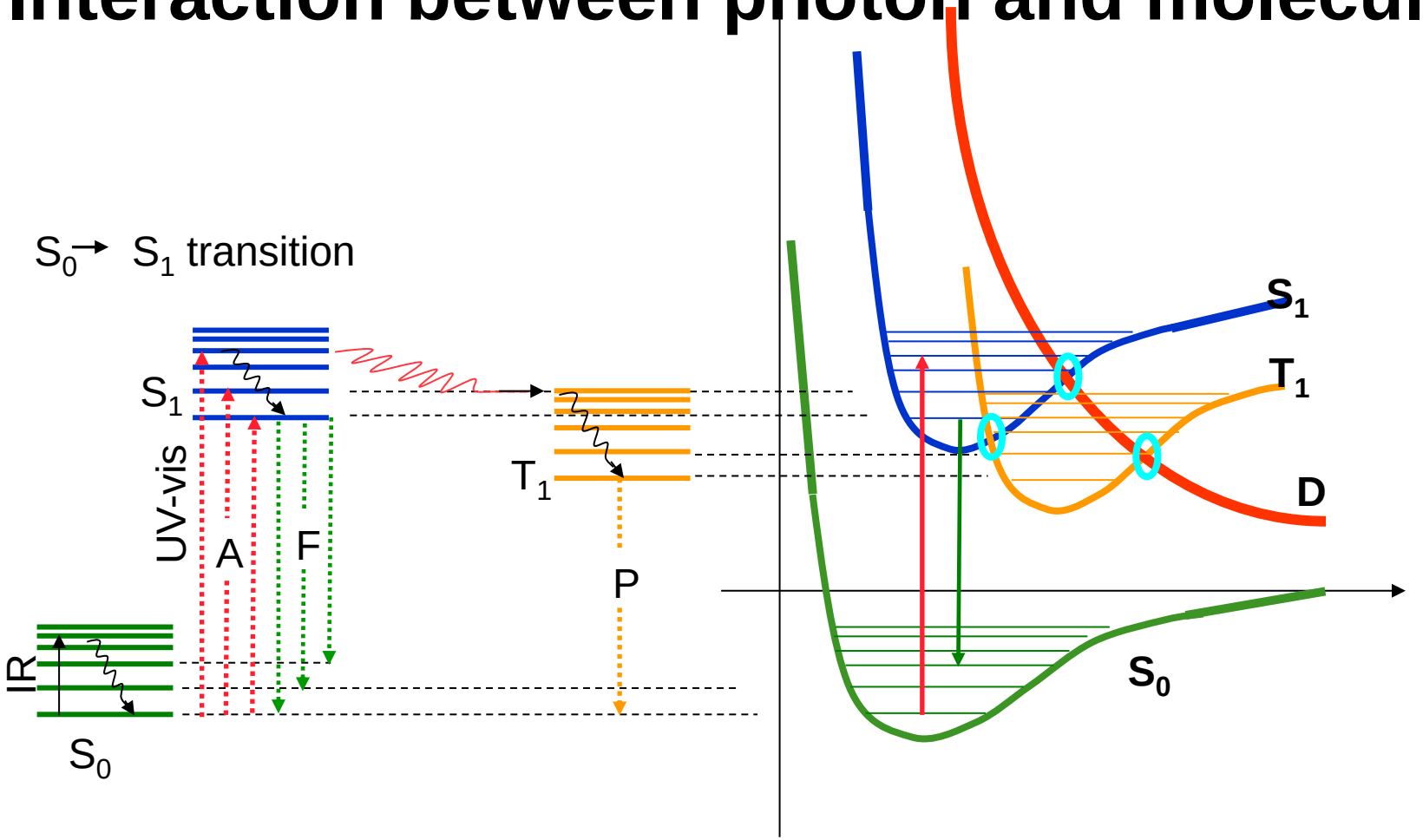
Transitions corresponding to:
e – electronic
v – vibrational
r – rotational

Energy S_1- S_0 ~ 80kcal/mol
335 kJ/mol

Energy v ~ 10kcal/mol
42 kJ/mol

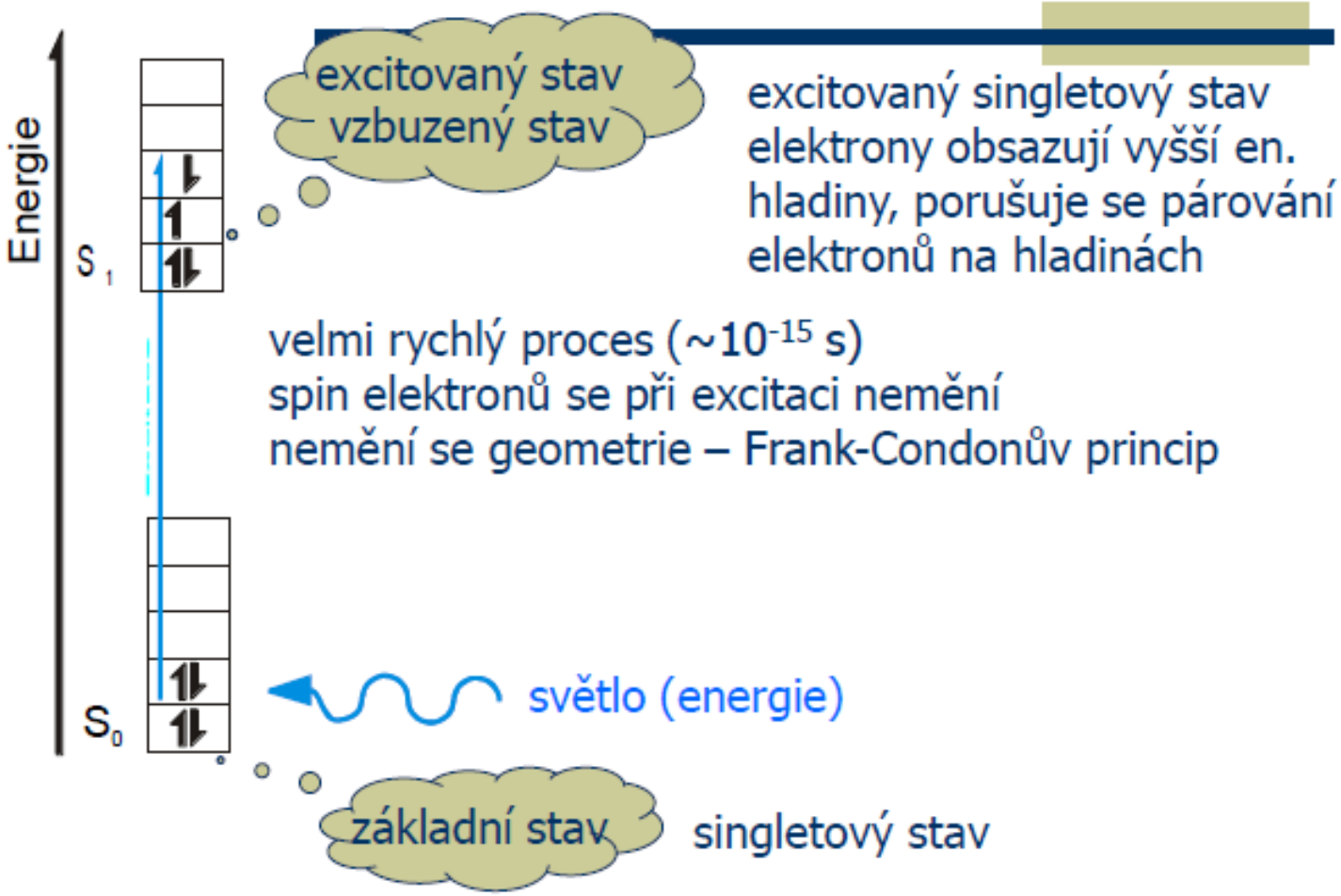
Energy r ~ 1 kcal/mol
4.2 kJ/mol

Interaction between photon and molecule



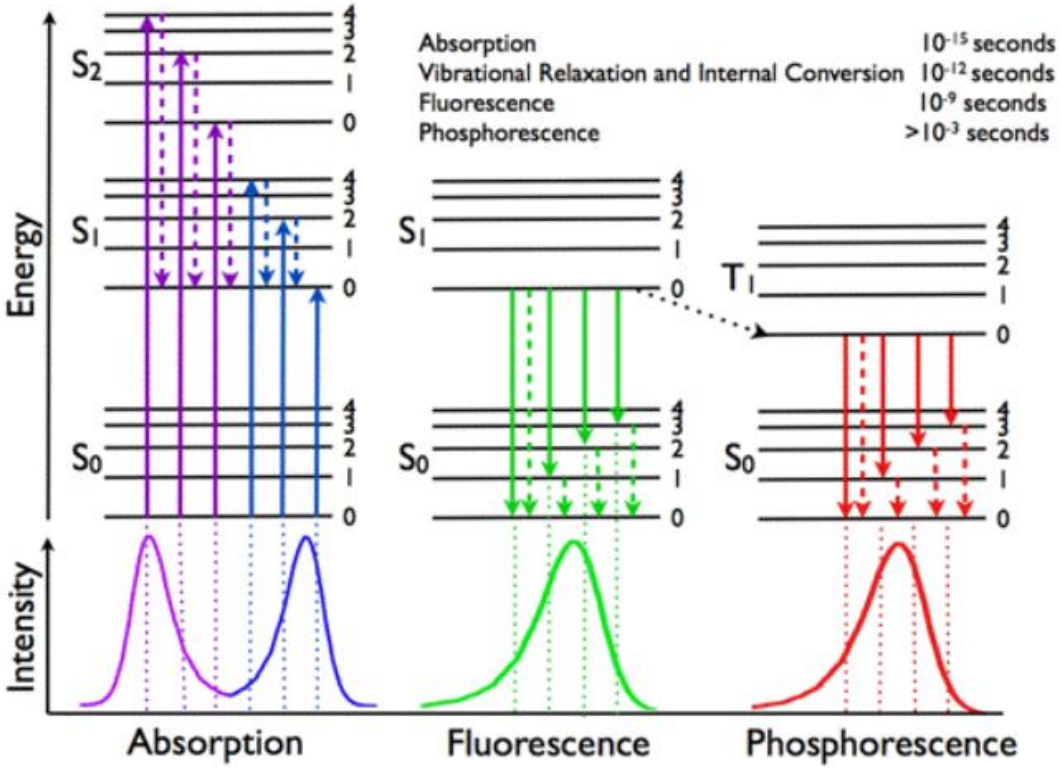
Absorption
Fluorescence
Phosphorescence

Absorpce fotonu

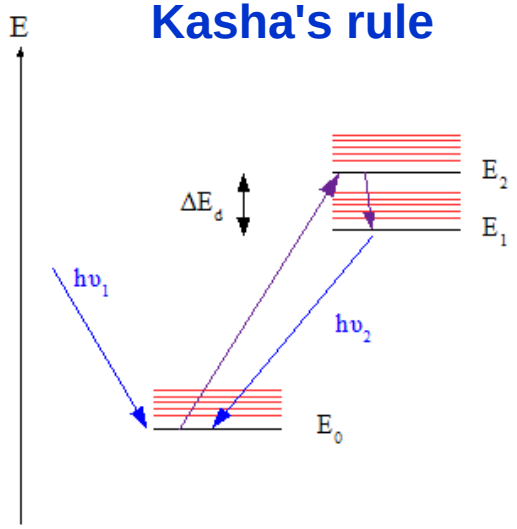


Interaction between photon and molecule

Jablonski diagram

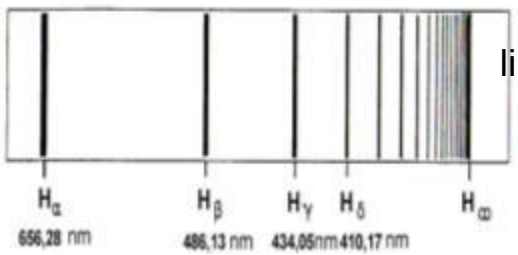


Kasha's rule

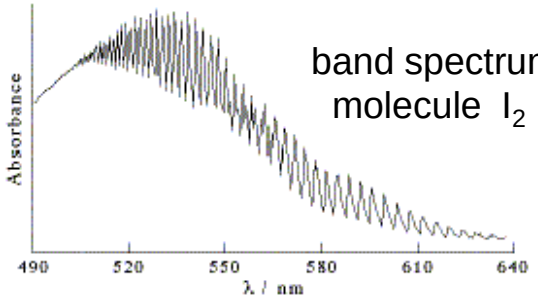


Kasha's rule is a principle in the photochemistry of electronically excited molecules. The rule states that photon emission (fluorescence or phosphorescence) occurs in appreciable yield only from the lowest excited state of a given multiplicity.

Electronic absorption spectra of small molecules



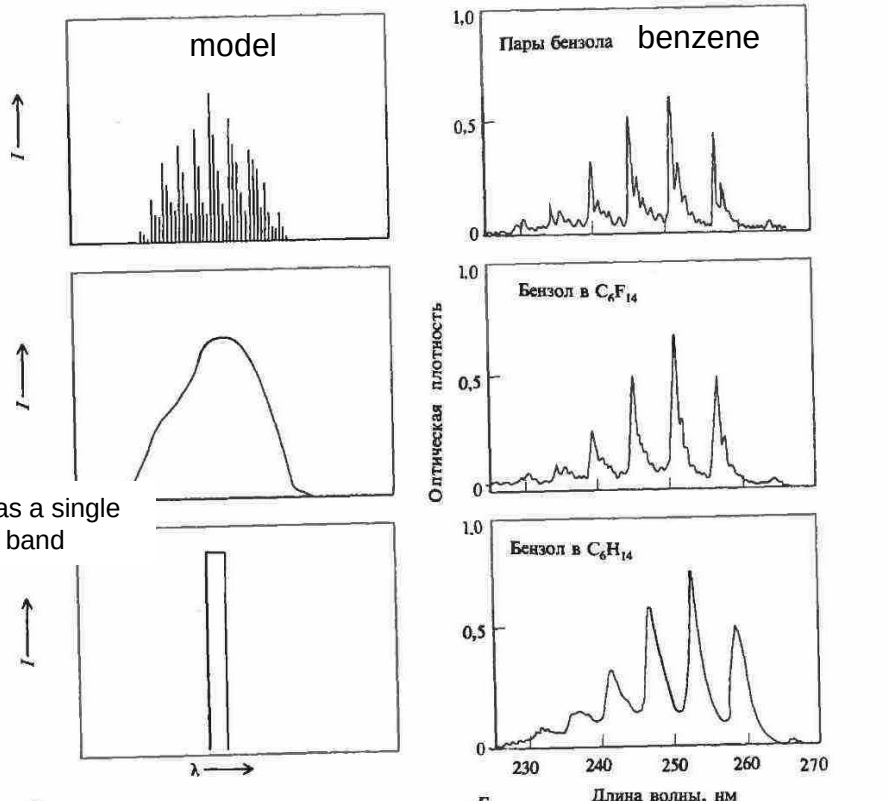
line spectrum
atom H



band spectrum
molecule I_2

magnitudes of transition dipoles

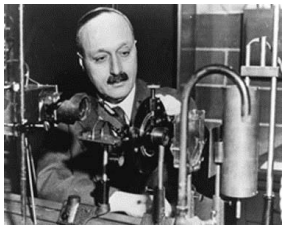
$$\langle S_1 vib, rot | \hat{\mu} | S_0 \rangle$$



absorption spectra of benzene showing solvent-induced broadening (gas, in solution, benzen in C6H14).

Band broadening: enviromental heterogeneity, Doppler shifts and other effects

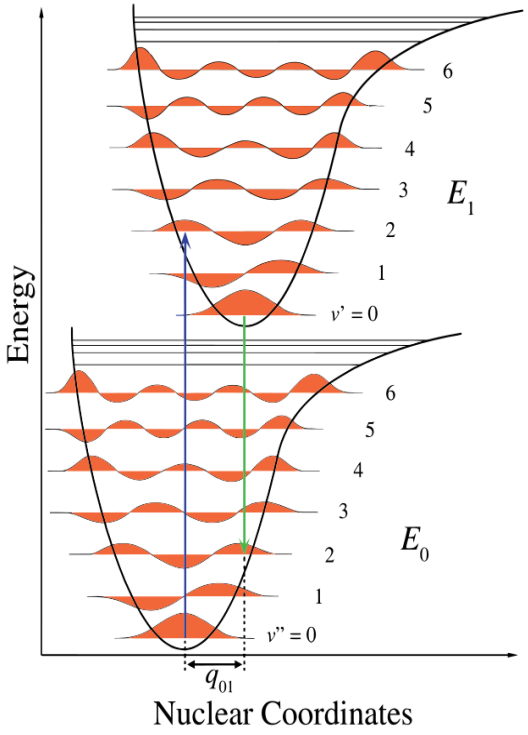
Franck-Condon (FC) Principle



James Franck (1882-1964)

Edward Uhler Condon 1902-1974

- The FC principle states that during an electronic transition, a change from one vibrational energy level to another will be more likely to happen if the two vibrational wave functions overlap more significantly.
- Classically, the FC principle is the approximation that an electronic transition is most likely to occur **without changes in the positions of the nuclei** in the molecular entity and its environment. The resulting state is called a FC state, and the transition involved, a **vertical transition**.
- The quantum mechanical formulation of this principle is that the intensity of a vibrational transition is proportional to the square of the overlap integral between the vibrational wave functions of the two states that are involved in the transition.



lazy nuclei

Since electronic transitions are very fast compared with nuclear motions, vibrational levels are favored when they correspond to a minimal change in the nuclear coordinates. The potential wells are shown favoring transitions between $v = 0$ and $v = 2$.

Absorbing species

1. excitation

$M + h\nu \longrightarrow M^*$

The lifetime of the excited species: 10^{-8} - 10^{-9} s

2. relaxation (conversion of the excitation energy to heat)

$M^* \longrightarrow M + \text{heat}$

The absorption of ultraviolet or visible radiation generally results from excitation of bonding electrons.

The electrons that contribute to absorption by a molecule are:

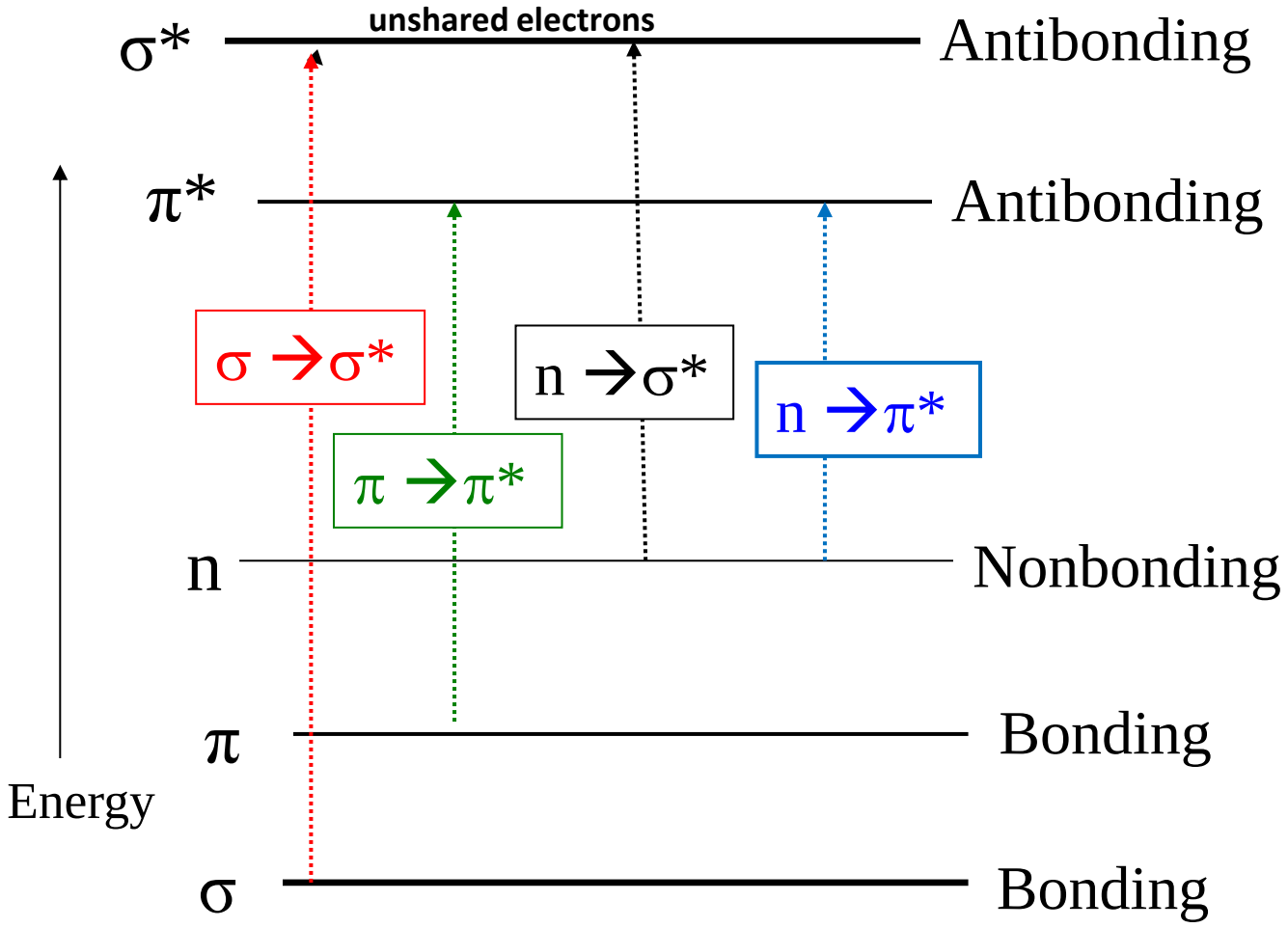
(1) those that participate directly in bond formation between atoms

(2) nonbonding or unshared outer electrons that are largely localized about such atoms as oxygen, the halogens, sulfur, and nitrogen. The molecular orbitals associated with single bonds are designated as sigma (σ) orbitals, and the corresponding electrons are σ electrons.

Energy

$\sigma \rightarrow \sigma^*$, $n \rightarrow \sigma^*$, $n \rightarrow \pi^*$, and $\pi \rightarrow \pi^*$

Methan 125 nm; 150-250nm ; most applications; 200-700nm; most applications; 200-700nm



Chromophores

Compound sample	Transition	λ_{max} (nm)
H_2O	$\sigma \rightarrow \sigma^*$	183
C-C a C-H, CH_4	$\sigma \rightarrow \sigma^*$	cca 170, 173
C-X, CH_3OH, CH_3NH_2, CH_3I	$n \rightarrow \sigma^*$	180-260, 187, 215, 258
C=C, $H_2C=CH_2$	$\pi \rightarrow \pi^*$	160-190, 162
$H_2C=CH-CH=CH_2$	$\pi \rightarrow \pi^*$	217
C=O, H−CH=O	$n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$	270, 170-200, 270, 185
$H_2C=CH-CH=O$	$n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$	328, 208
C=N	$n \rightarrow \sigma^*$, $n \rightarrow \pi^*$	190, 300
N=N	$n \rightarrow \pi^*$	340
C=S	$n \rightarrow \pi^*$	500
NO_2	$n \rightarrow \pi^*$	420-450
N=O	$n \rightarrow \pi^*$	630-700

Chromophores

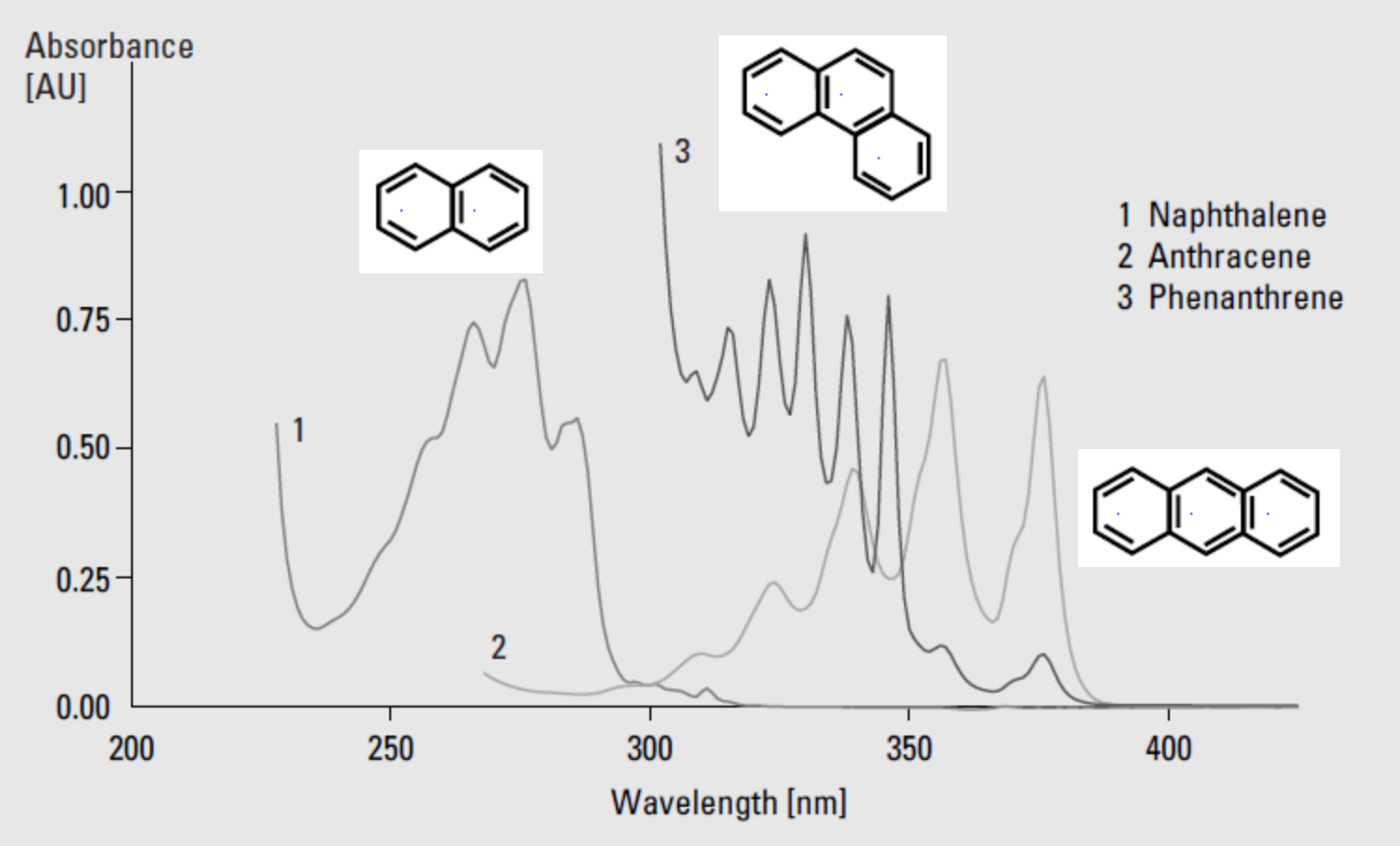
Absorption Characteristics of Some Common Chromophores

Chromophore	Example	Solvent	λ_{max}, nm	ε_{max}	Transition Type
Alkene	$C_6H_{13}CH{=}CH_2$	*n*-Heptane	177	13,000	$\pi \rightarrow \pi^*$
Alkyne	$C_5H_{11}C{\equiv}C{-}CH_3$	*n*-Heptane	178	10,000	$\pi \rightarrow \pi^*$
			196	2000	—
			225	160	—
Carbonyl	$CH_3\overset{\overset{O}{\|}}{C}CH_3$	*n*-Hexane	186	1000	$n \rightarrow \sigma^*$
			280	16	$n \rightarrow \pi^*$
	$CH_3\overset{\overset{O}{\|}}{C}H$	*n*-Hexane	180	large	$n \rightarrow \sigma^*$
			293	12	$n \rightarrow \pi^*$
Carboxyl	CH_3COOH	Ethanol	204	41	$n \rightarrow \pi^*$
Amido	$CH_3\overset{\overset{O}{\|}}{C}NH_2$	Water	214	60	$n \rightarrow \pi^*$
Azo	$CH_3N{=}NCH_3$	Ethanol	339	5	$n \rightarrow \pi^*$
Nitro	CH_3NO_2	Isooctane	280	22	$n \rightarrow \pi^*$
Nitroso	C_4H_9NO	Ethyl ether	300	100	—
			665	20	$n \rightarrow \pi^*$
Nitrate	$C_2H_5ONO_2$	Dioxane	270	12	$n \rightarrow \pi^*$

Effect of Conjugation of Chromophores

1,3-butadiene, $CH_2{=}CHCH{=}CH_2$: a strong absorption band that is displaced to a longer wavelength by 20 nm compared with the corresponding peak for an unconjugated diene

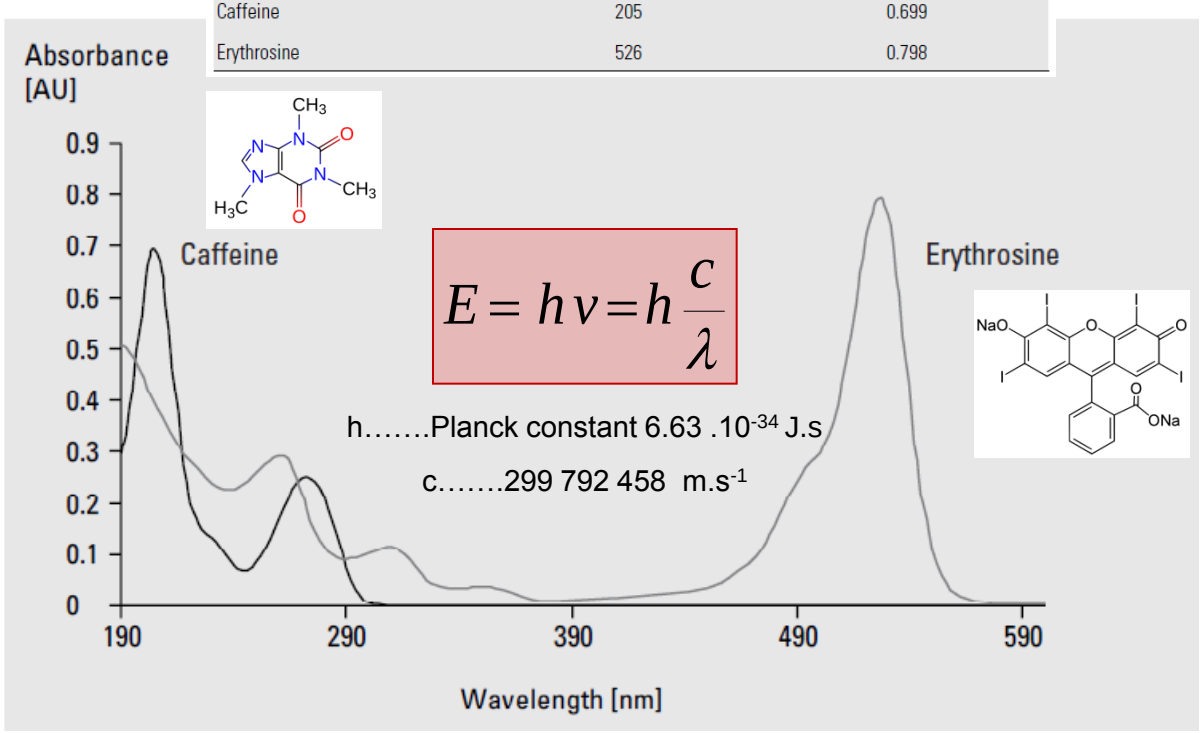
Chromophores and conjugated double bonds



- Not only the kind of chromophore is important; the benzene-rings are chromophores with π–π* transitions but conjugated double bonds have the spectral effect. The environment of the chromophores or the combination with other chromophores also have a strong influence on the position and values of absorbance bands
- From naphthalene to anthracene there is an increasing conjugation of the π bonds. The spectral region of absorbance changes as well as the intensity of absorbance and the form of the absorbance bands
- Anthracene and phenanthrene have the same chemical formula but their spectra are different because their structure and hence the electronic environment of the π bonds are different.

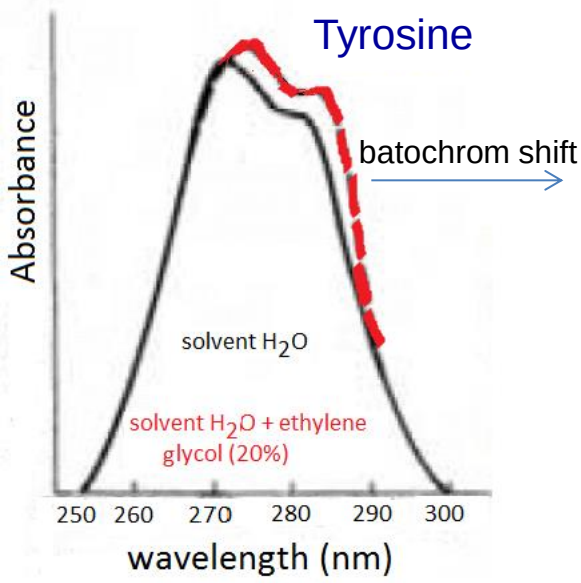
Energies of Light at the Absorbance Maxima

Compound	λ_{max} [nm]	Absorbance [AU]
Caffeine	205	0.699
Erythrosine	526	0.798



Compound	λ_{max} [nm]	Energy [J]
Caffeine	205	$9.69 \cdot 10^{-19}$
Erythrosine	526	$3.78 \cdot 10^{-19}$

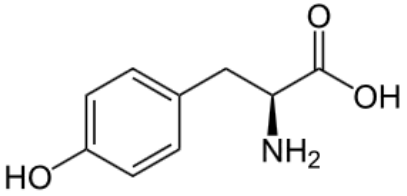
Solvation effect



solvation influences the distribution of energy levels of the base and the excited state (effect of surface active substance)

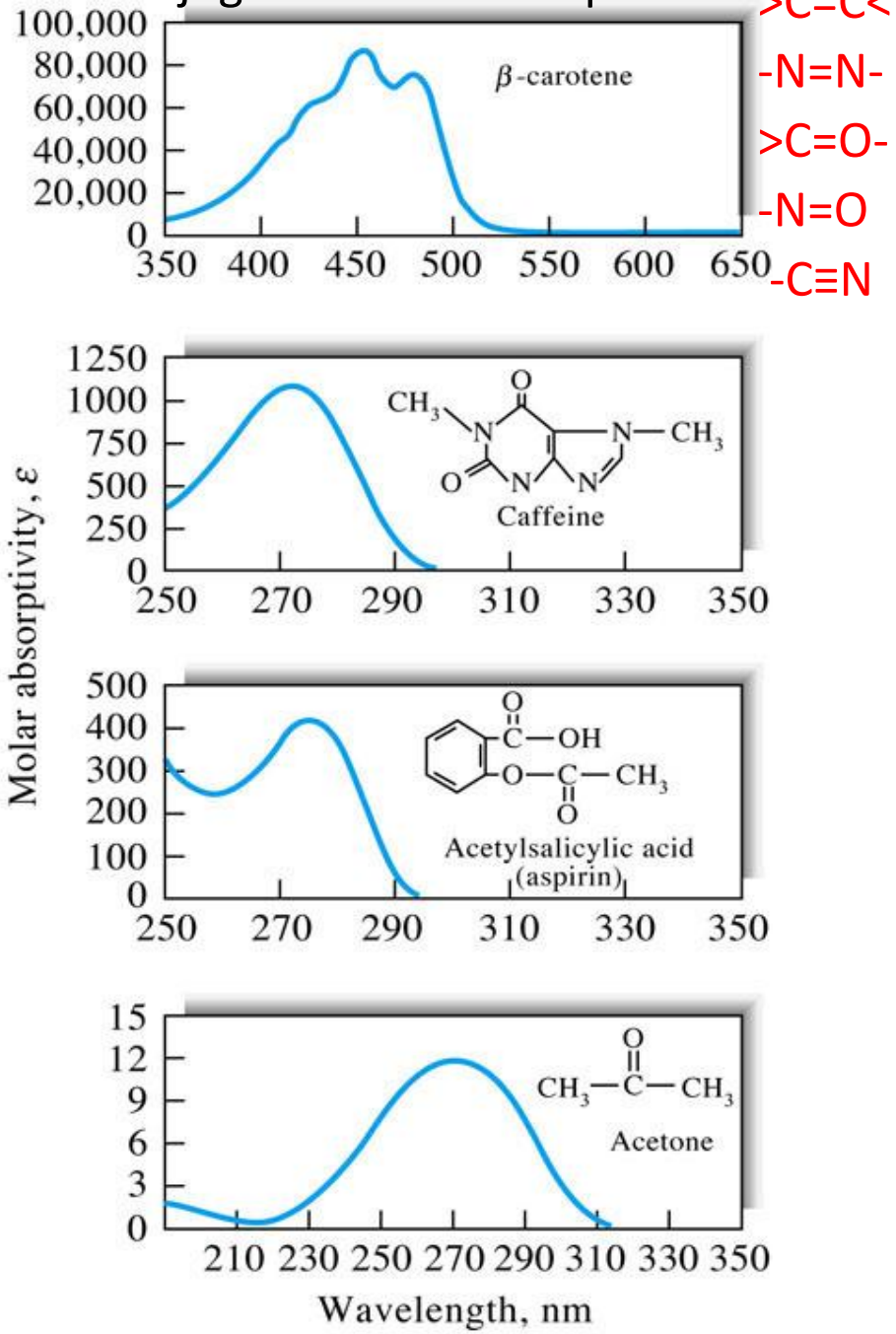
There is a relationship between the wavelengths of the absorbance maxima and the polarity of the solvent used (permitivity)

similar effect of pH: protonation-deprotonation

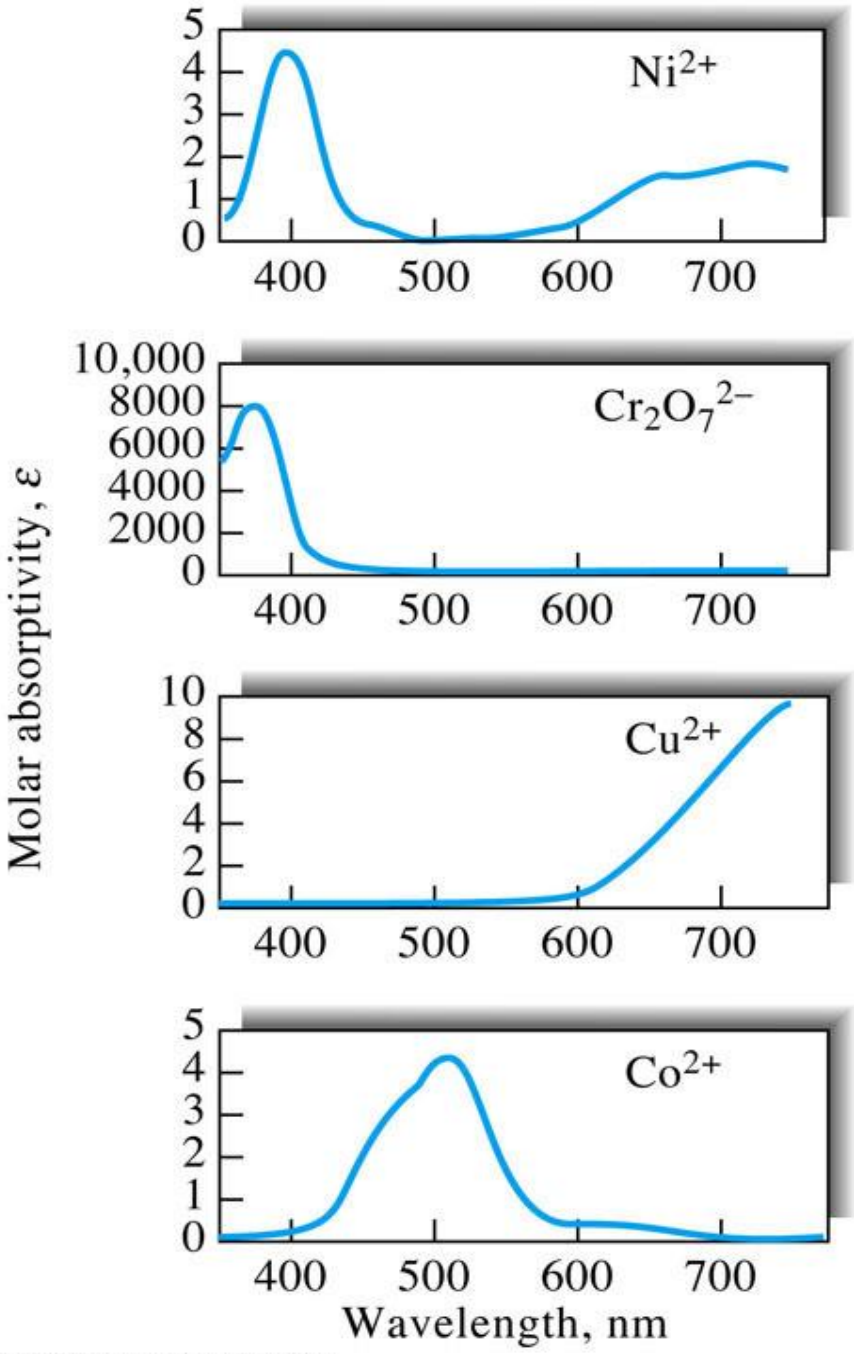


conjugation of chromophores

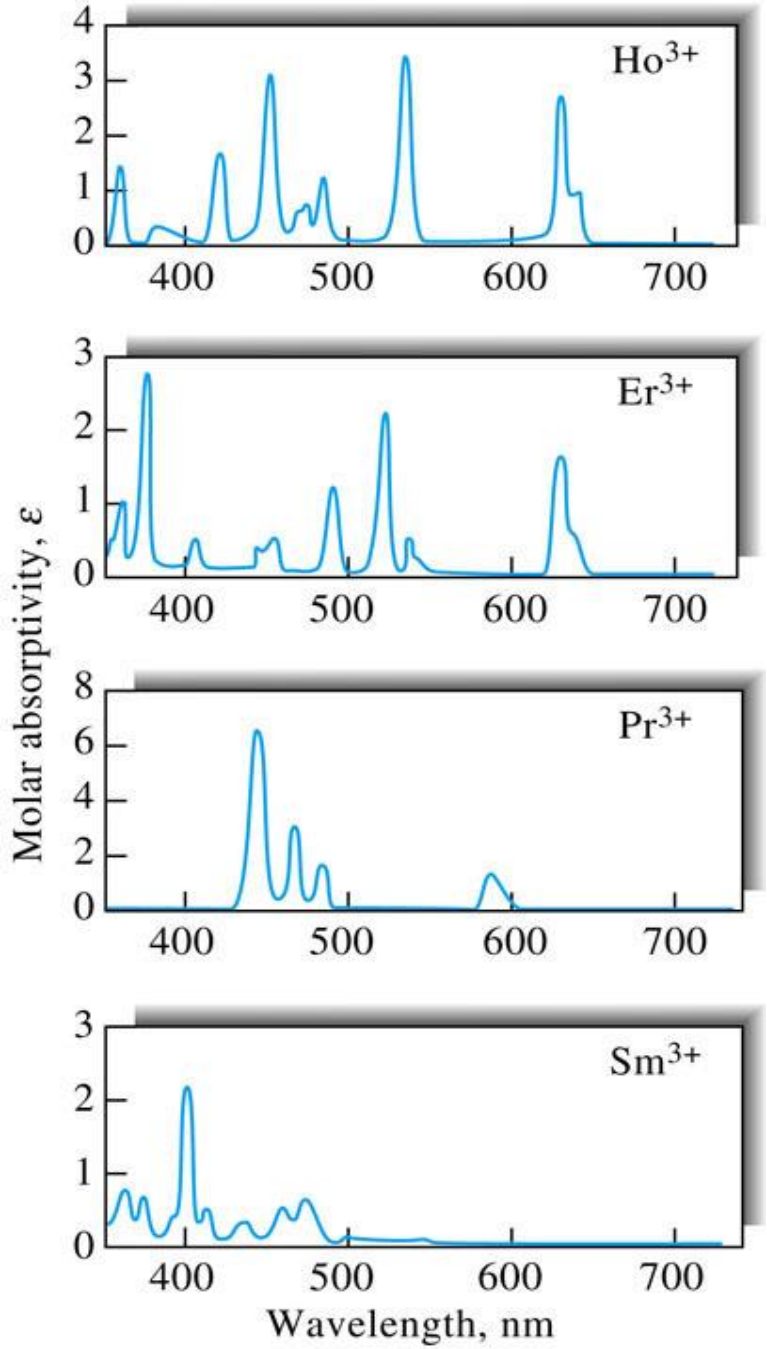
>C=C<
-N=N-
>C=O-
-N=O
-C≡N



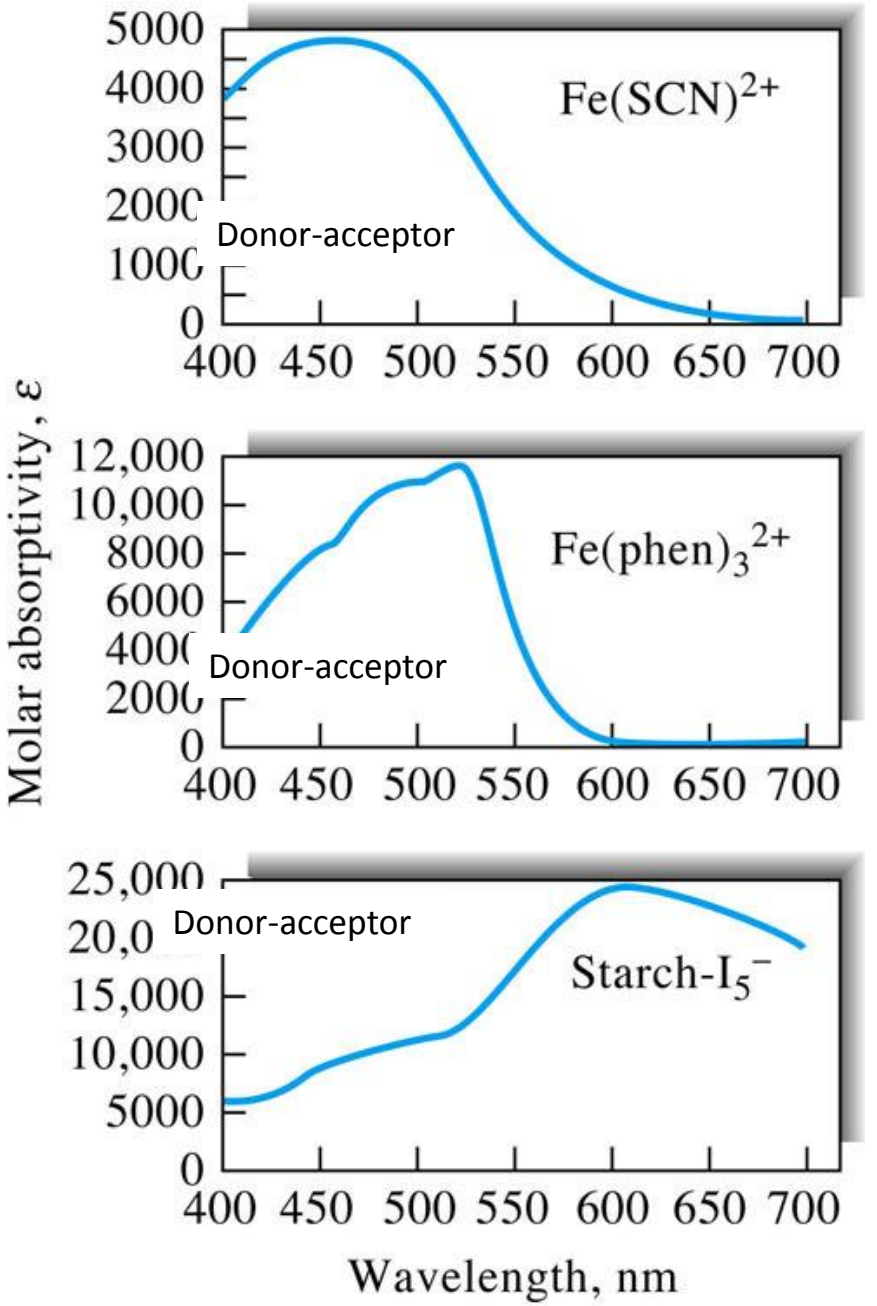
d and **f** electrons



Lanthanide and Actinide Ions

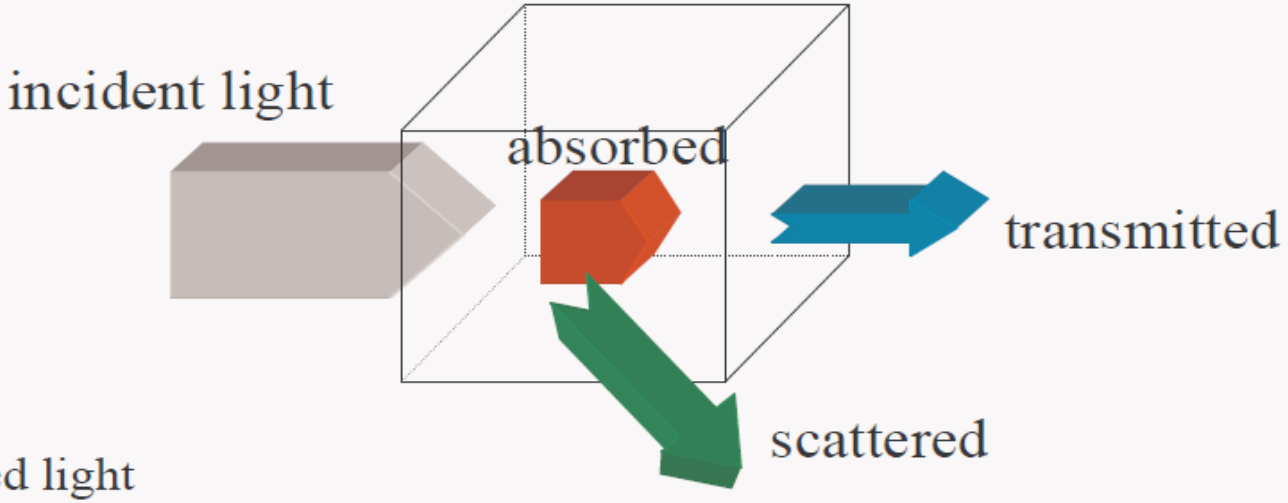


Charge-Transfer Absorption



Light

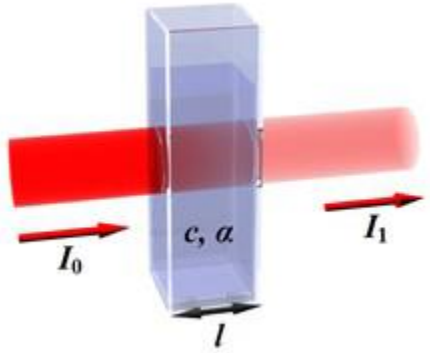
- Consider a beam of light on a material
  - It can be scattered, absorbed, or transmitted



- Transmitted light
  - Light emerges propagating in the same direction as the incident light
- Absorbed light
  - Energy from light is absorbed in the volume of the material
- Scattered light
  - Light emerges in a different direction from the incident light

Beer–Lambert–Bouguer law

the extinction coefficient



$$-\frac{dI}{I} = C\varepsilon' dl$$

Absorbance A

ε' the molar extinction coefficient = f (λ or ω)

$$-\int_{I_0}^{I}\frac{dI}{I} = \int_{0}^{l} C\varepsilon' dl \longrightarrow ln\frac{I_0}{I} = C\varepsilon' l \longrightarrow A(\lambda) \equiv log\frac{I_0}{I} = C\varepsilon(\lambda)l$$

$$\varepsilon = \varepsilon' / 2.302$$

- low concentrations < 10^{-2} M
- monochromatic light
- stabillity of sample

Transmittance T

$$T(\lambda) \equiv \frac{I}{I_0}$$

$$I = I_0 e^{-\varepsilon'(\lambda)l}$$

$$A(\lambda) = log\frac{I_0}{I} = log\frac{I_0}{I_0 e^{-\varepsilon'(\lambda)l}} = log\, e^{\varepsilon'(\lambda)l} = \varepsilon'(\lambda)l\, log\, e = 0.4343\varepsilon'(\lambda)l = 0.1886\varepsilon(\lambda)l$$

the most accurate measurements of A

DNA bases

$$\varepsilon_{max}\ [\,mol^{-1}cm^{-1}\,] \cong 10^4$$

biopolymers

problems with ε: high and non-correct due to not known molecular weight ……the average ε per residue, for ODN = phosphate residues

ε molar and residue extinction is often implicit

biopolymers

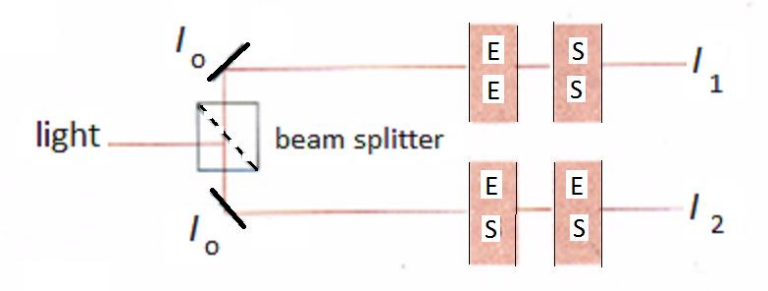
Sample: $\log I_S = \log I_0 - A_S$

Reference: $\log I_R = \log I_0 - A_R$

Double beam spectrophotometer

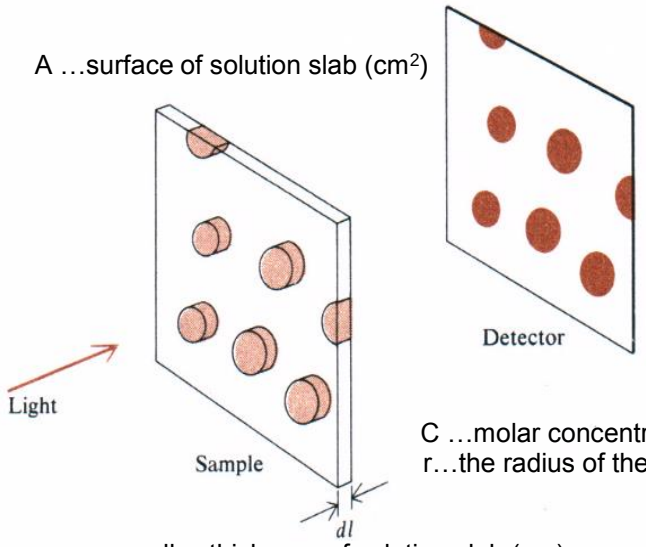
$$\log \frac{I_R}{I_S} = A_S - A_R$$

Differential spectrophotometry



$$\log \frac{I_2}{I_1} = A_1 - A_2 = 2\left[(\varepsilon_E C_E + \varepsilon_S C_S) - (\varepsilon_E C'_E + \varepsilon_S C'_S + \varepsilon_{ES} C'_{ES})\right] l$$

the extinction coefficient and cross sections (molecular size)



A …surface of solution slab (cm^2)

C …molar concentration of solute (M)

r…the radius of the solute molecule

dl… thickness of solution slab (cm)

$$f_{max} = \left(\pi r^2 CAN_{0dl} / 1{,}000\right) / Adl = \pi r^2 CN_0 / 1{,}000$$

the number of solute molecules in the slab

the number of solute molecules per cm^2

f_{max} the fraction of the cross-sectional area of the slab occupied by solute molecules

P… the probability that light impinging on a molecule is absorbed

$f_{max}P$ the fraction of incident light absorbed

$$\sigma = P\pi r^2$$

σ... *the cross section of the molecule*

$$-\frac{dI}{I} = C\varepsilon' dl$$

$$ln\frac{I_0}{I} = C\varepsilon' l$$

$$A(\lambda) \equiv log\frac{I_0}{I} = C\varepsilon(\lambda) l$$

$$\frac{dI}{I} = \left(\sigma CN_0 / 1{,}000\right) dl$$

$$C\varepsilon' dl = \left(\sigma CN_0 / 1{,}000\right) dl$$

$$\varepsilon' = \sigma N_0 / 1{,}000 \qquad \sigma = P\pi r^2$$

$$\varepsilon = \varepsilon' / 2.302$$

σ... aromatic ring $\approx 1\text{Å}$

$$\boxed{\varepsilon = P\pi r^2 N_0 / 2{,}303}$$

the extinction coefficient in calculating molecular properties

$$-\frac{dI(\nu)}{dt} = h\nu(N_a B_{ab} - N_b B_{ba})I(\nu)$$

Avogadro´s number

the number of excited molecules (N_b) is negligible

$$-\frac{dI(\nu)}{dt} = (h\nu N_0 B_{ab}/1{,}000)I(\nu)$$

the rate of energy uptake for a one-molar solution

$$dI(\nu) = \left(\frac{1}{c}\right)\left(\frac{dI(\nu)}{dt}\right)dl = \left(\frac{h\nu N_0 B_{ab}}{1{,}000\,c}\right)I(\nu)\,dl$$

$$-\frac{dI}{I} = C\varepsilon' dl$$

$$B_{ab} = 1{,}000\,\varepsilon' c / N_0 h\nu$$

for both states, **integration** over a band frequencies

$$B_{ab} = \frac{1{,}000\,c}{N_0 h}\int \frac{\varepsilon'}{\nu} d\nu$$

Dipole strength

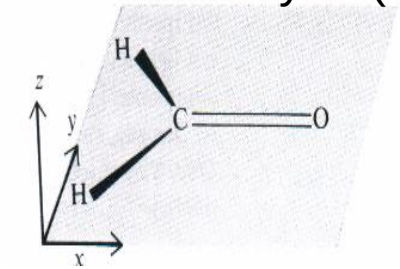
$$B_{ab} = (2/3)(\pi/\hbar^2)\left|\langle \psi_b | \hat{\mu} | \psi_a \rangle\right|^2$$

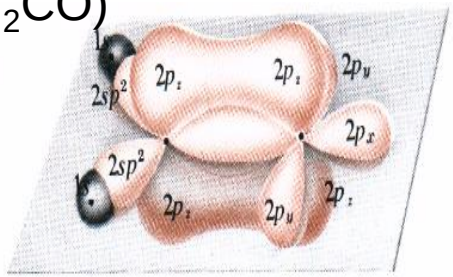
$$\varepsilon = \varepsilon' / 2.302$$

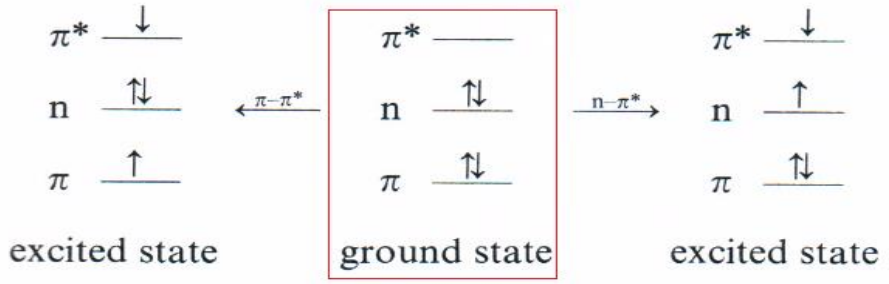
$$D_{ab} \equiv \left|\langle \psi_b | \hat{\mu} | \psi_a \rangle\right|^2 = 0.00918 \int \frac{\varepsilon}{\nu} d\nu \quad (debye)^2$$

Spectral properties of a simple molecule

Formaldehyde (H_2CO)







H……..1s

C……..$1s^2\ 2s^2\ 2p_y\ 2p_x$ hybridization…. $1s^2\ (2sp^2)^3\ 2p_z$

O……..$1s^2\ 2s^2\ 2p_y^2\ 2p_x\ 2p_z$

The absorption intensity

the transition dipole moment

$$\langle i|\hat{\mu}|f\rangle$$

initial final

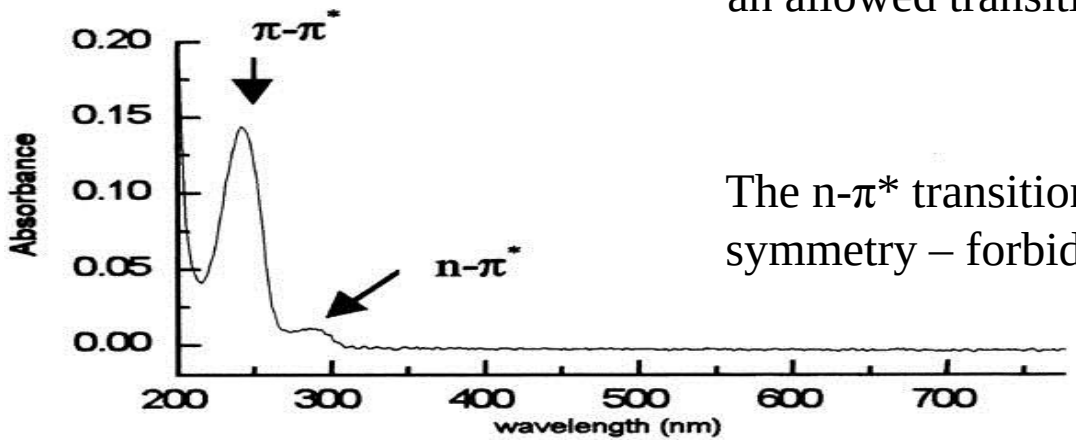
$$\langle \pi|\hat{\mu}|\pi^* \rangle \qquad \langle n|\hat{\mu}|\pi^* \rangle$$

$$\psi_{\pi} = 2p_z(C) + 2p_z(O)$$

$$\psi_{\pi^*} = 2p_z(C) - 2p_z(O)$$

The π-π* transition is called an allowed transition

The n-π* transition is called a symmetry – forbidden transition



Instrumentation - spectrophotometry

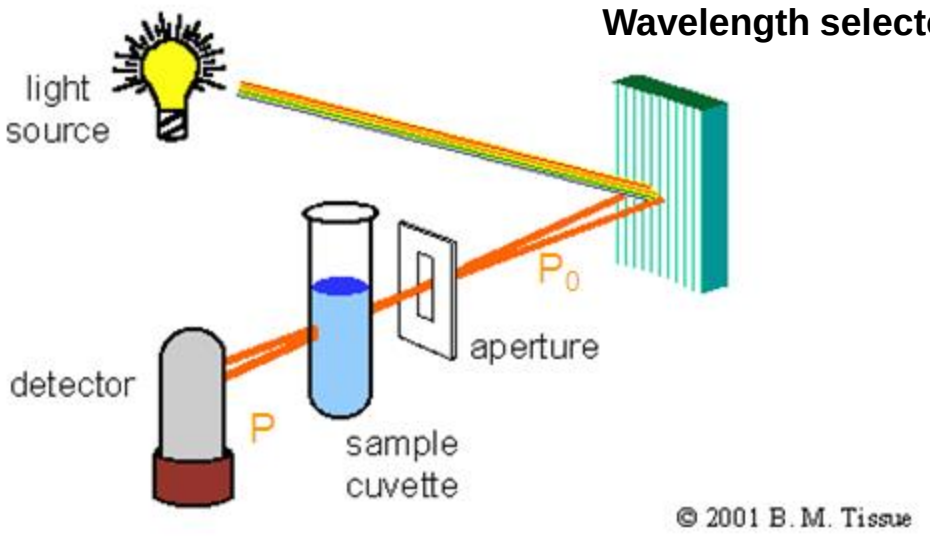
- **Spectrophotometer**
- **Single** and **double** beam instruments

  Components of optical instruments

  1. Sources
  2. Wavelength selectors (filters, monochromators)
  3. Sample containers
  4. Detectors
  5. Readout devices
- **Applications of Spectrophotometry**

  Spectrophotometry is more suited for quantitative analysis rather than qualitative one

Instrumentation (Spectrophotometers)

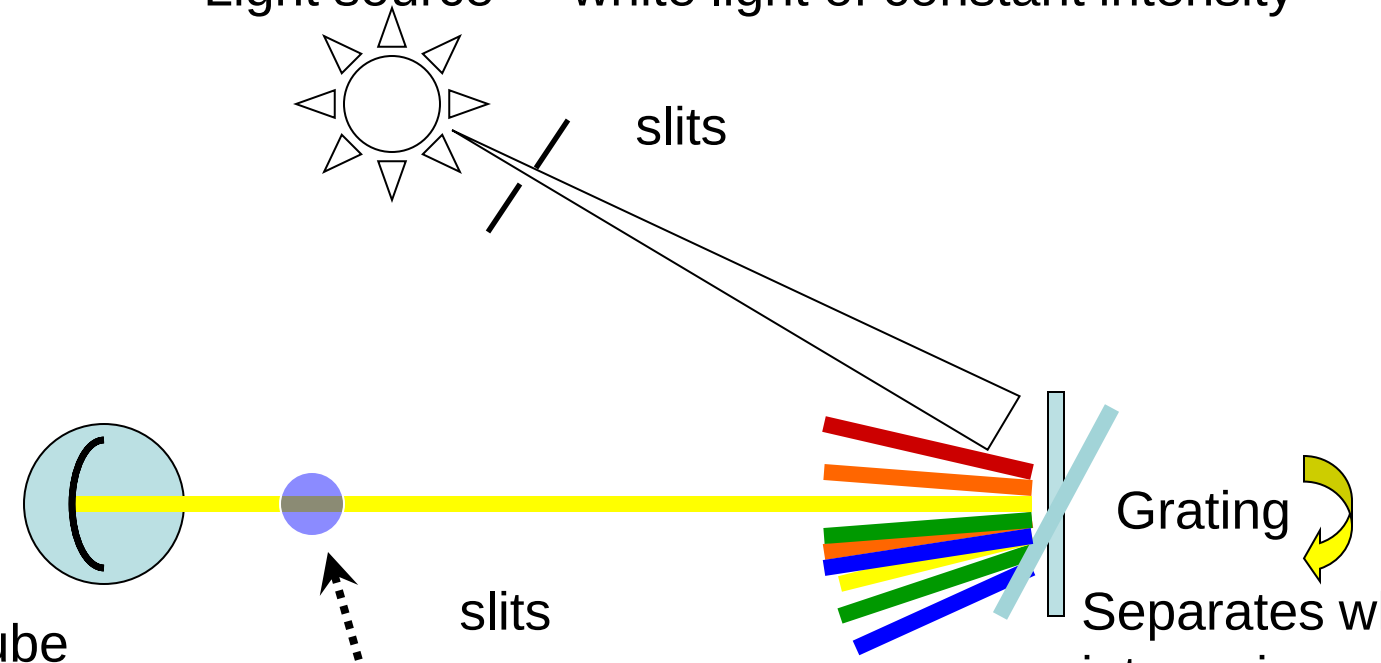


A single beam spectrophotometer

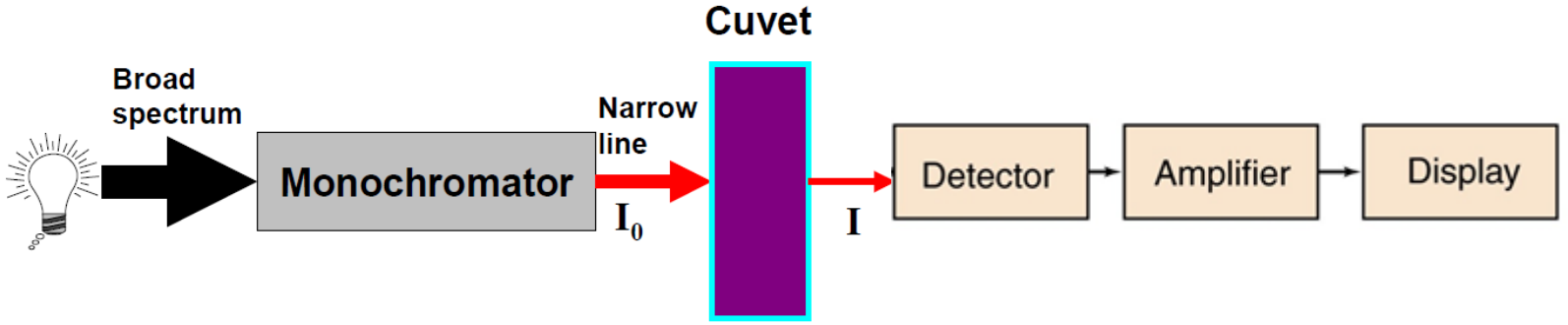
The above essential features of a spectrophotometer shows that polychromatic light from a **source** separated into narrow band of wavelength (nearly monochromatic light) by a **wavelength selector**, passed through the **sample compartment** and the transmitted intensity, P, after the sample is measured by a **detector**

In a **single beam instrument**, the light beam follows ***a single path*** from the source, to the monochromator, to the sample cell and finally to the detector

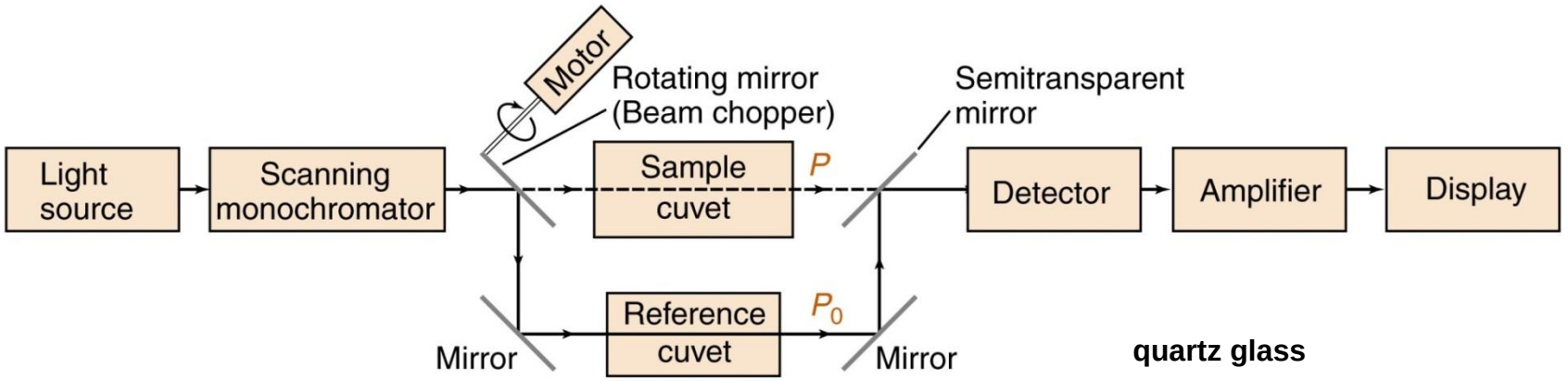
The components of a single beam spectrophotometer



Single beam spectrophotometer



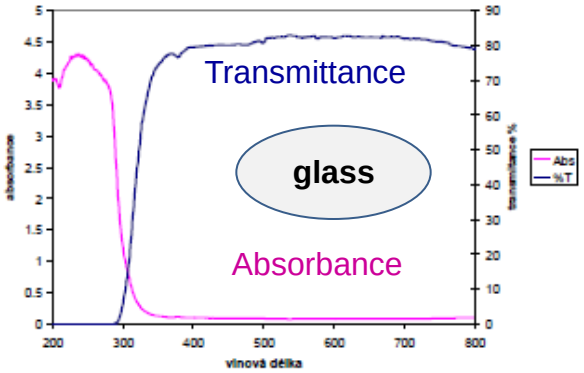
Double Beam Spectrophotometer



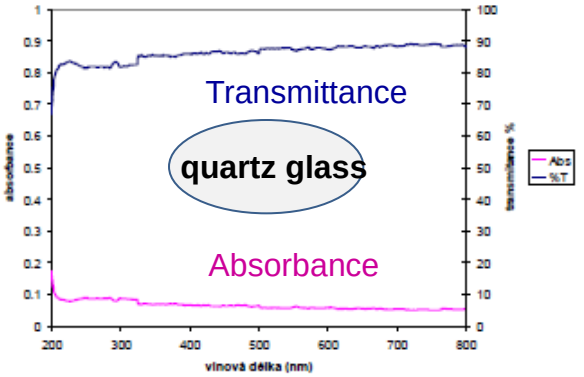
Range: 200 – 1100 nm

Occuracy: 2 nm

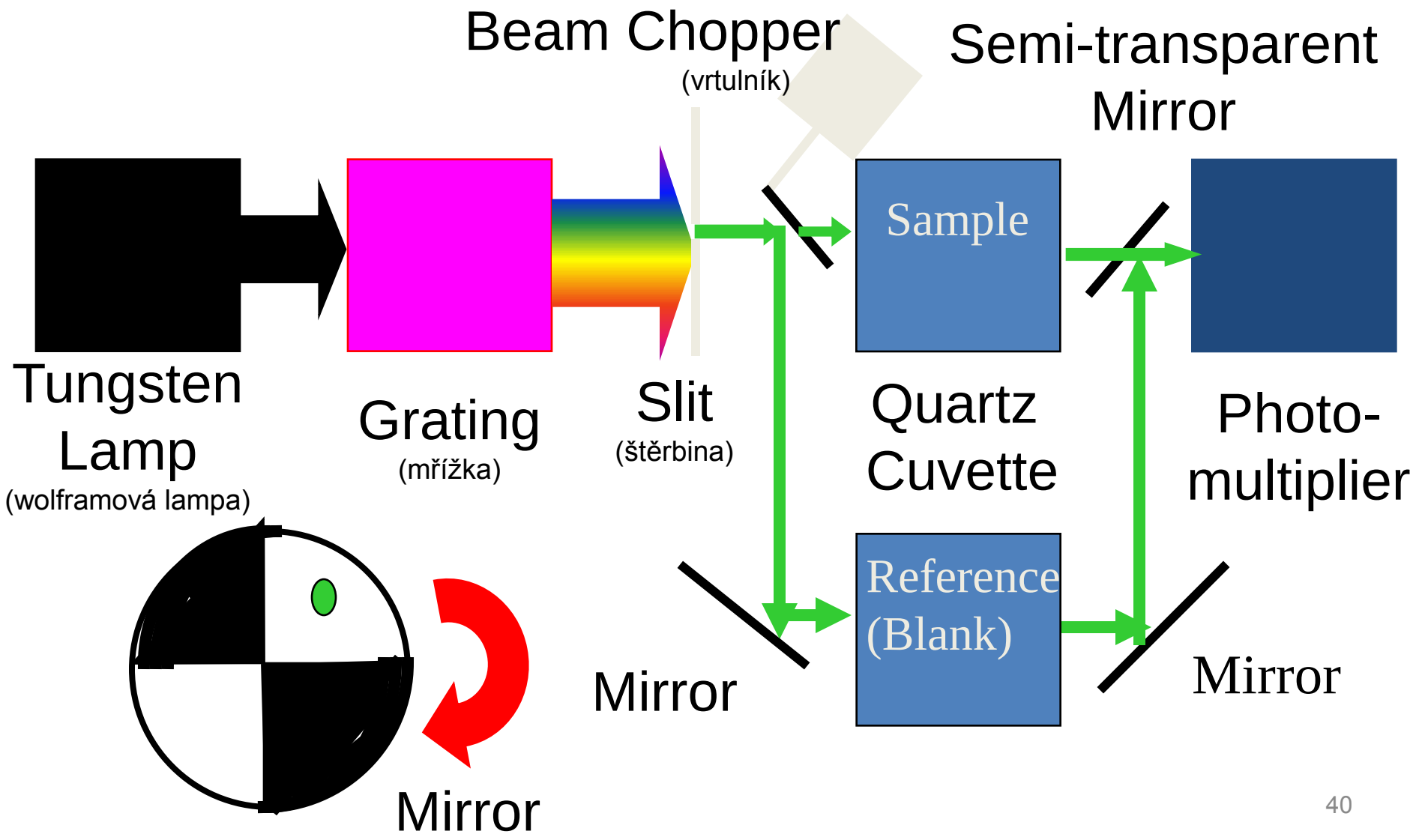
Absorption of air <200 nm



quartz glass

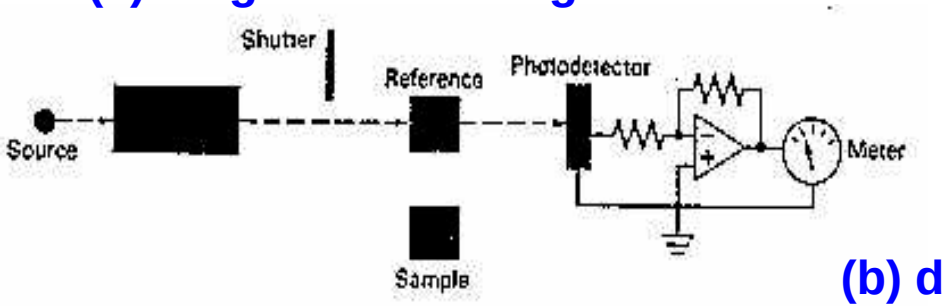


Double Beam Spectrophotometer

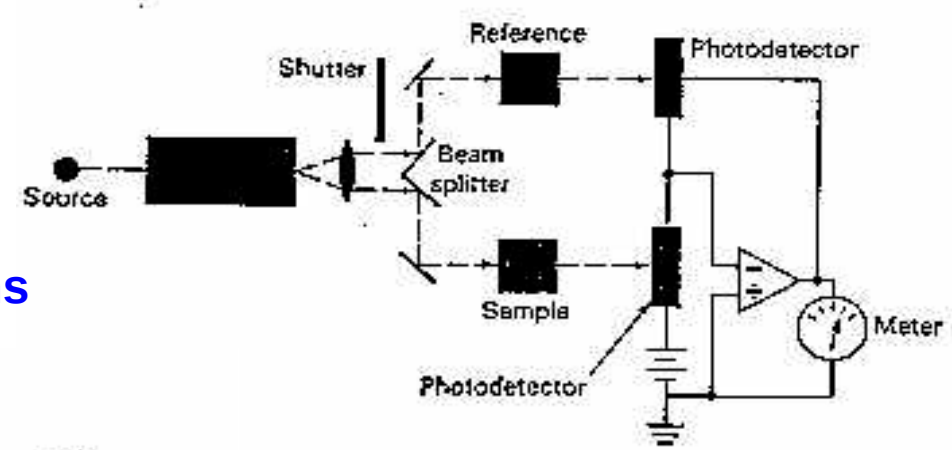


Single Beam vs. Double Beam

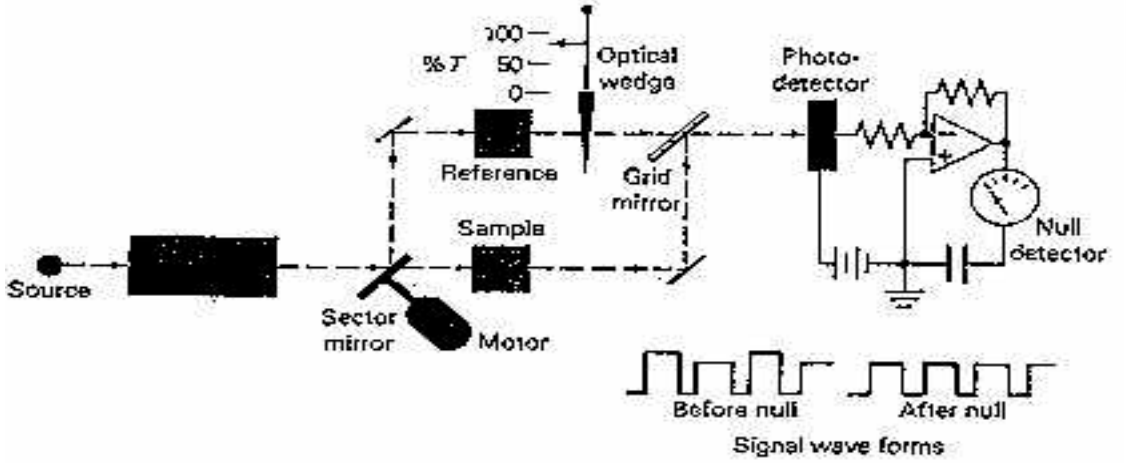
(a) single-beam design



(b) dual channel design with beams separated in space but simultaneous in time



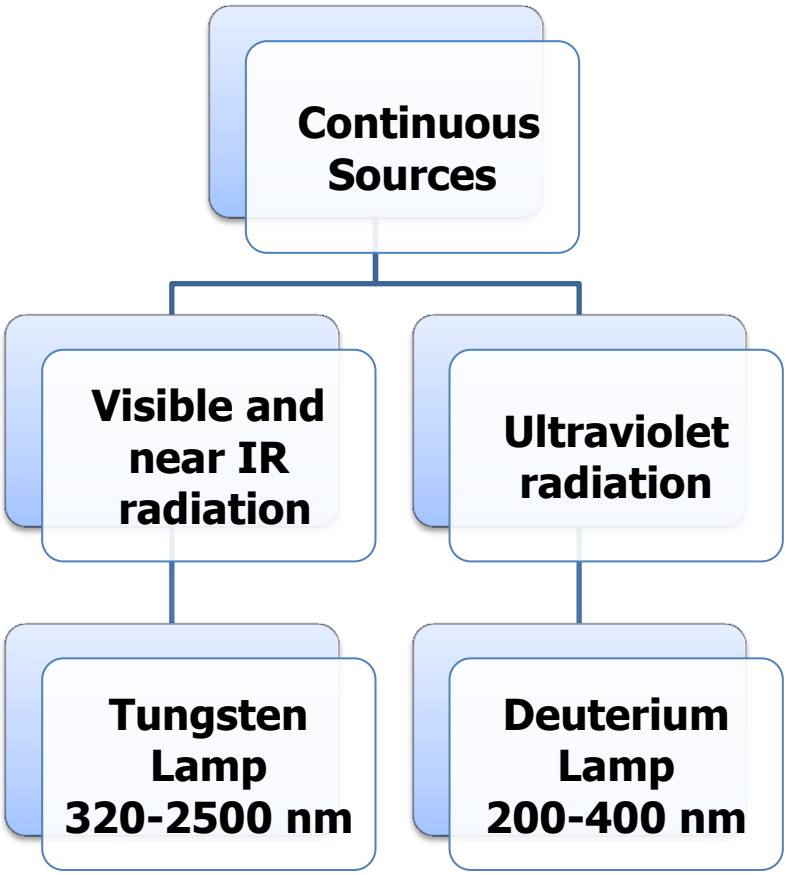
(c) double-beam design in which beams alternate between two channels."



Light sources

Sources used in UV-Vis Spectrophotometers are continuous sources.

- **Continuous sources** emit radiation of all wavelengths within the spectral region for which they are to be used.
- **Sources of radiation** should also be stable and of high intensity.



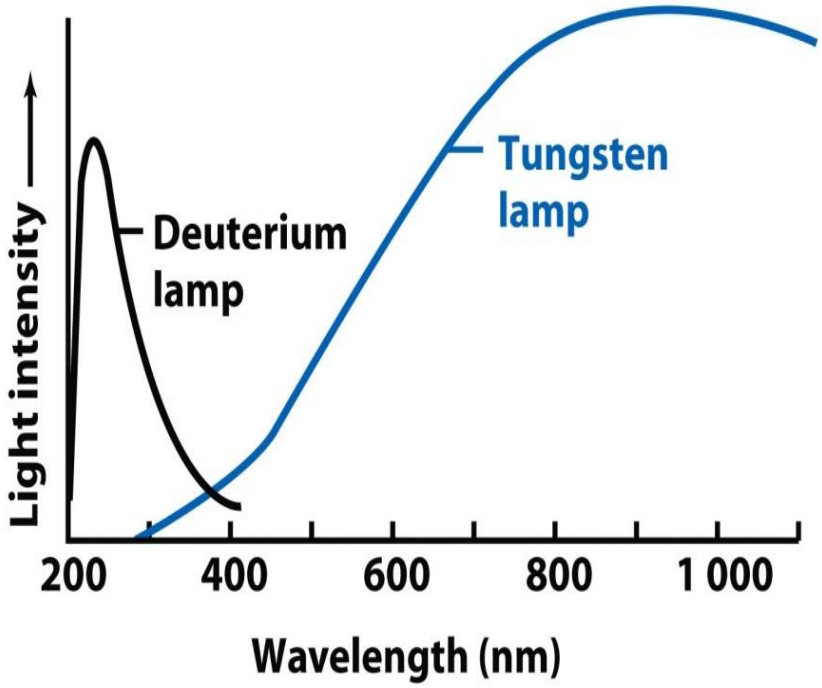


Figure 20-3
Quantitative Chemical Analysis, Seventh Edition

Light sources

Black-body radiation for Vis and IR but not UV

- a *tungsten lamp* is an excellent source of black-body radiation
- operates at 3000 K
- produces λ from 320 to 2500 nm

For UV:

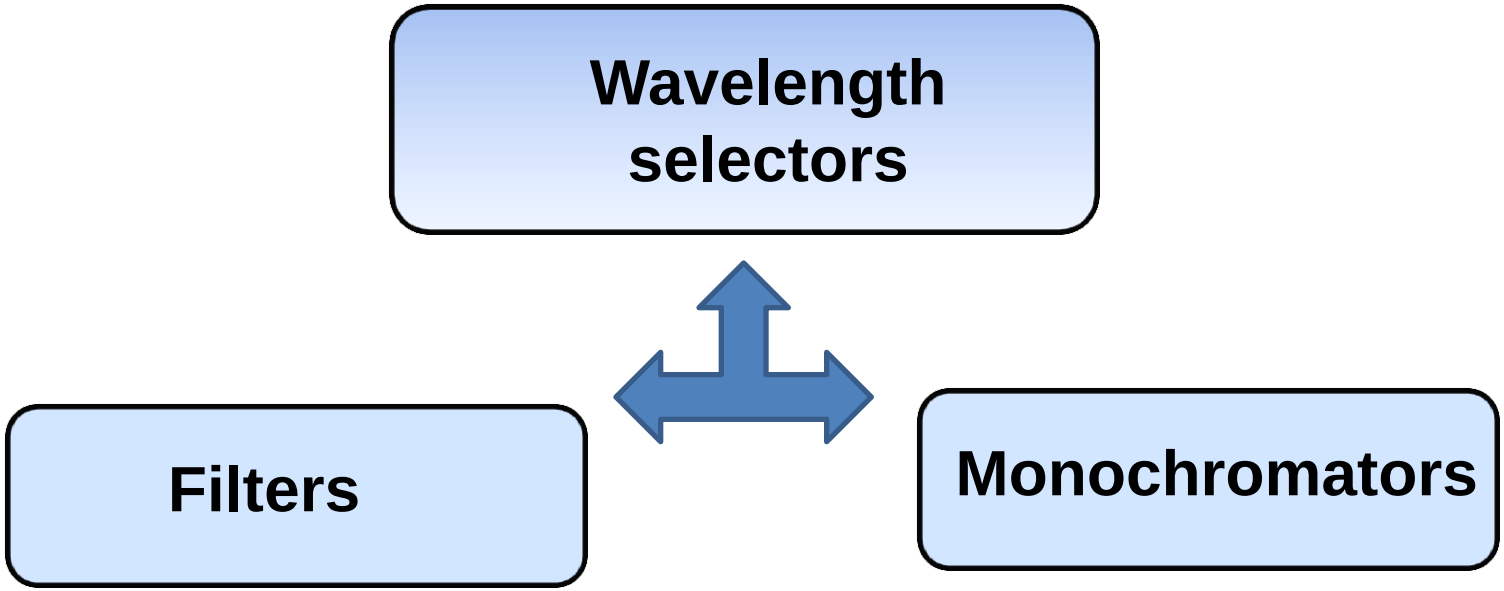
- a common lamp is a deuterium arc lamp
- electric discharge causes D_2 to dissociate and emit UV radiation (160 – 325 nm)
- other good sources are: Xe (250 – 1000 nm) or Hg (280 – 1400 nm)

Wavelength Selectors

Ideally the output of a wavelength selector would be a radiation of a **single wavelength**.

No real wavelength selector is ideal, usually a **band** of radiation is obtained.

The **narrower** this bandwidth is , the **better** performance of the instrument.



i- Filters

- **Filters permit certain bands of wavelength (bandwidth of ~ 50 nm) to pass through.**
- **The simplest kind of filter is absorption filters , the most common of this type of filters is colored glass filters.**
- **They are used in the visible region.**
- **The colored glass absorbs a broad portion of the spectrum (complementary color) and transmits other portions (its color).**

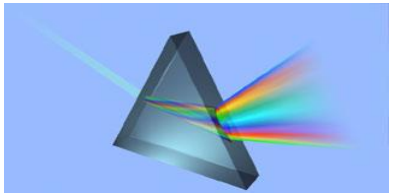
Disadvantage

- **They are not very good wavelength selectors and can't be used in instruments utilized in research.**
- **This is because they allow the passage of a broad bandwidth which gives a chance for deviations from Beer's law.**
- **They absorb a significant fraction of the desired radiation.**

Monochromators

Early spectrophotometers used prisms

- quartz for UV
- glass for vis and IR



These are now superseded by:

Diffraction gratings:

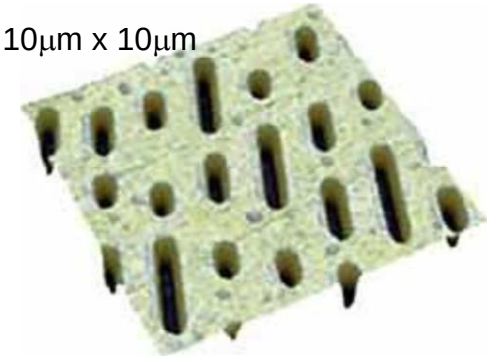
- made by drawing lines on a glass with a diamond stylus

  ca. 20 grooves mm^{-1} for far IR

  ca. 6000 mm^{-1} for UV/vis

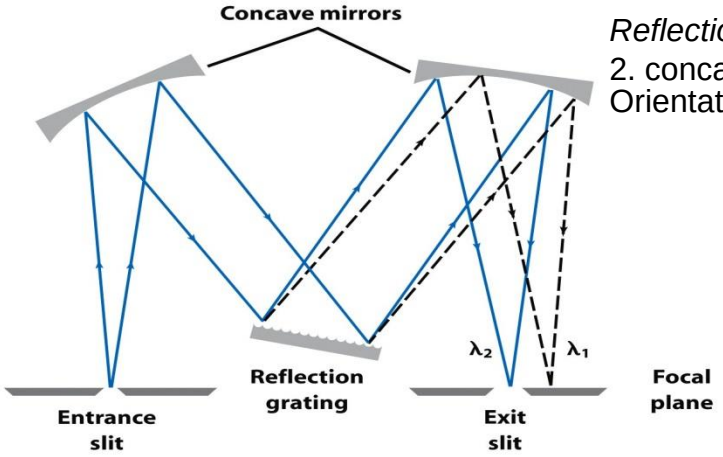
- can use plastic replicas in less expensive instruments

Think of diffraction on a CD



What is the purpose of concave mirrors?

The light is *collimated* the first concave mirror

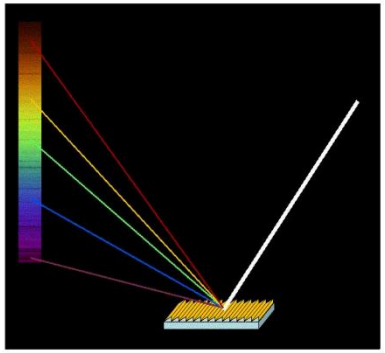


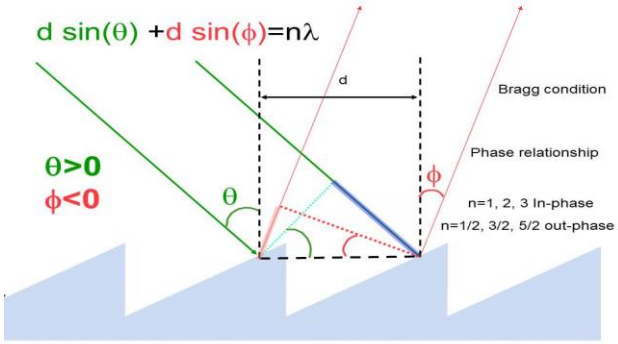
Reflection grating diffracts different wavelengths at different angles

2. concave mirror focuses each wavelength at different point of *focal plane*

Orientation of the reflection grating directs only one narrow band to exit slit

Polychromatic radiation enters

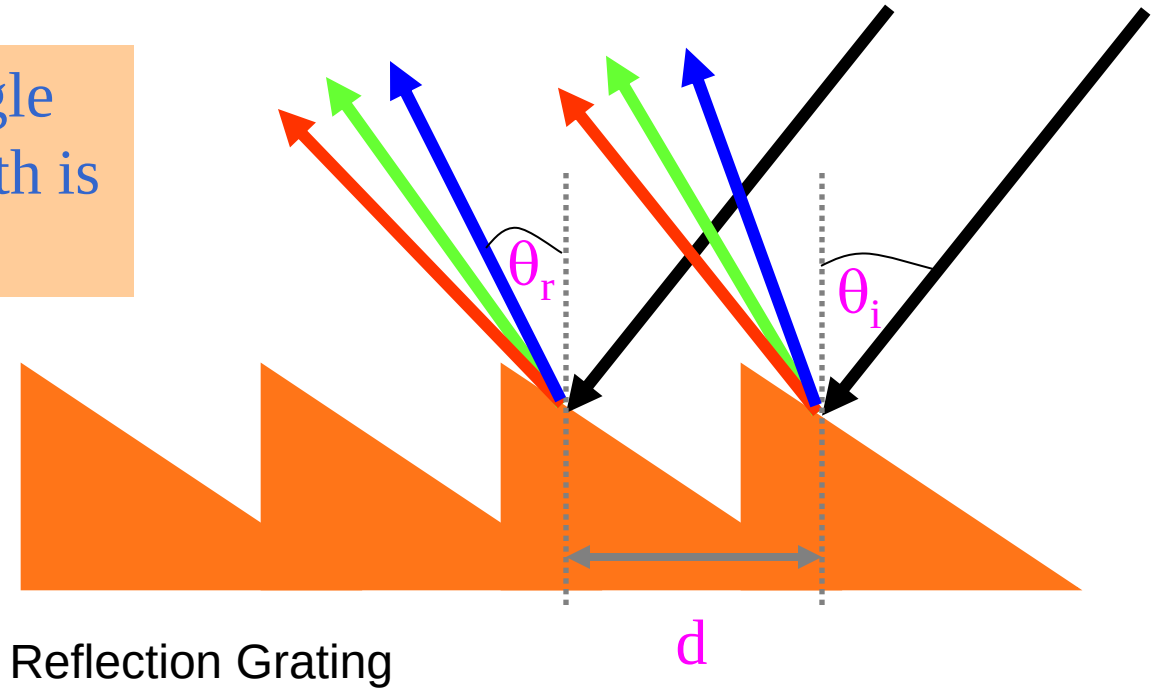




Echellette Grating equation

- $n\lambda = d(\sin\theta_i + \sin\theta_r)$ where n = 1, 2, 3,….
- Since incident angle θ_i = constant; therefore $\lambda \propto \theta_r$

For each reflection angle θ_r, a certain wavelength is observed



Reflection Grating

Note: For more detail see Skoog text book p. 159-160

Monochromators: reflection grating

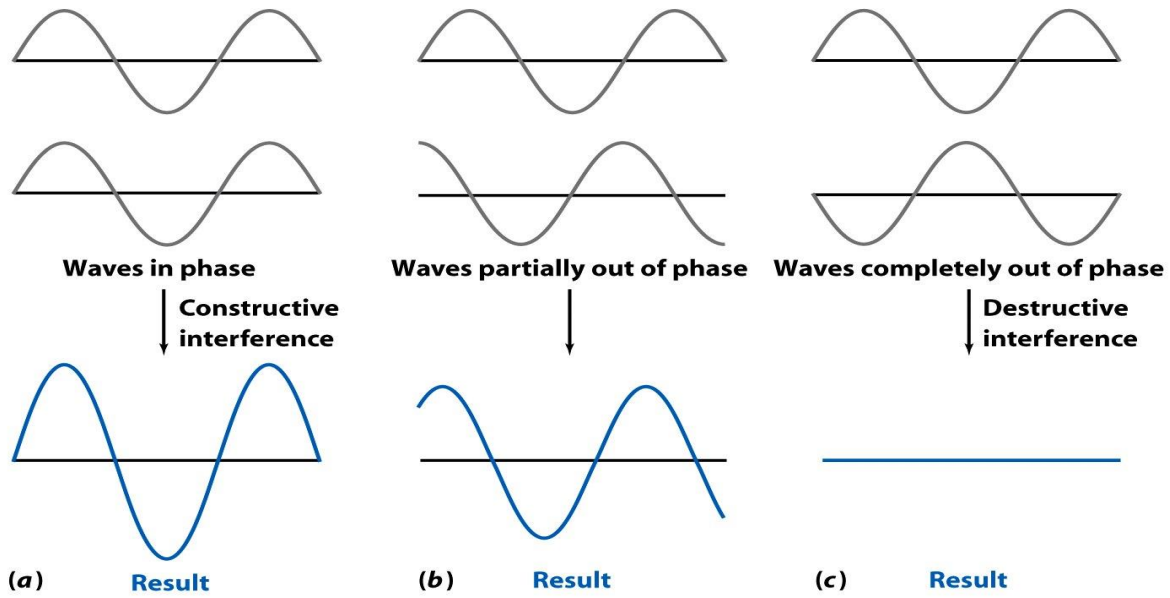
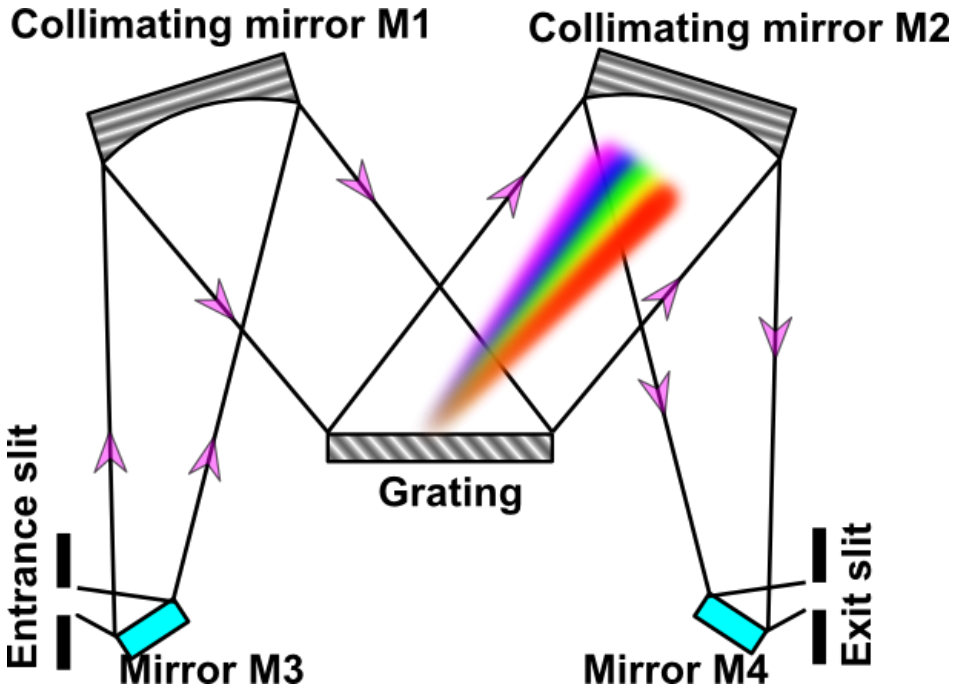


Figure 20-7
Quantitative Chemical Analysis, Seventh Edition

- Each wavelength is diffracted off the grating at a different angle
- Angle of deviation of diffracted beam is λ dependent → diffraction grating separates the incident beam into its constituent wavelengths components
- **Groove dimensions and spacings are on the order of the wavelength in question**

ii- Monochromators

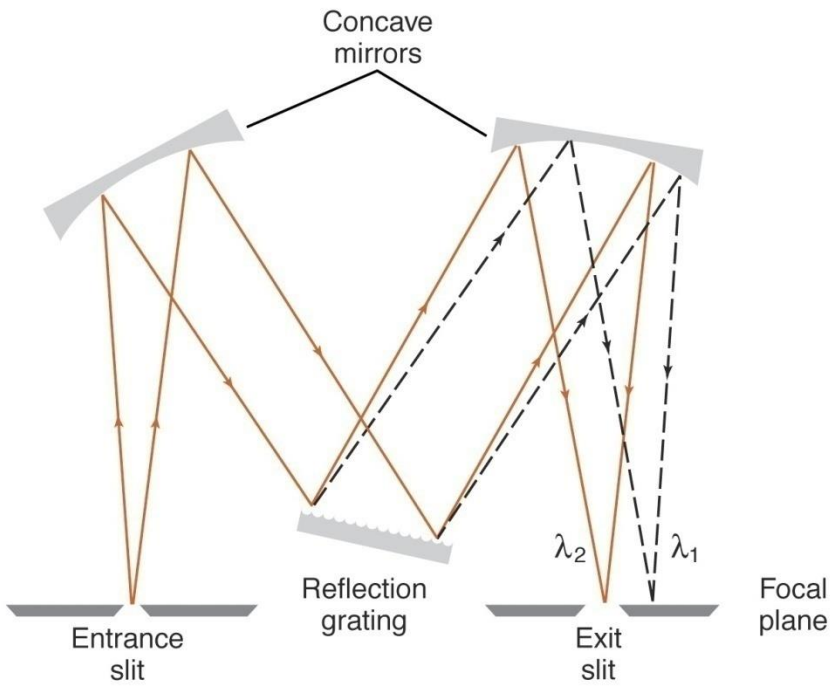
- They are used for **spectral scanning** (varying the wavelength of radiation over a considerable range).
- They can be used for **UV/Vis** region.
- All monochromators are similar in mechanical construction.
- All monochromators employ **slits, mirrors, lenses, gratings or prisms.**



Grating monochromators

Reflection grating

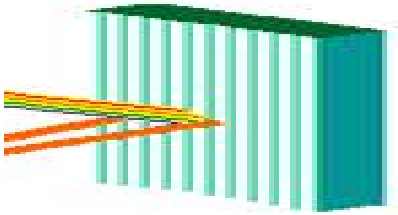
- Polychromatic radiation from the entrance slit is collimated (made into beam of parallel rays) by a concave mirrors
- These rays fall on a reflection grating, whereupon **different wavelengths are reflected at different angles.**
- The orientation of the reflection grating directs only one narrow band wavelengths, λ_2, to the exit slit of the mono-chromator
- Rotation of the grating allows different wavelengths, λ_1, to pass through the exit slit



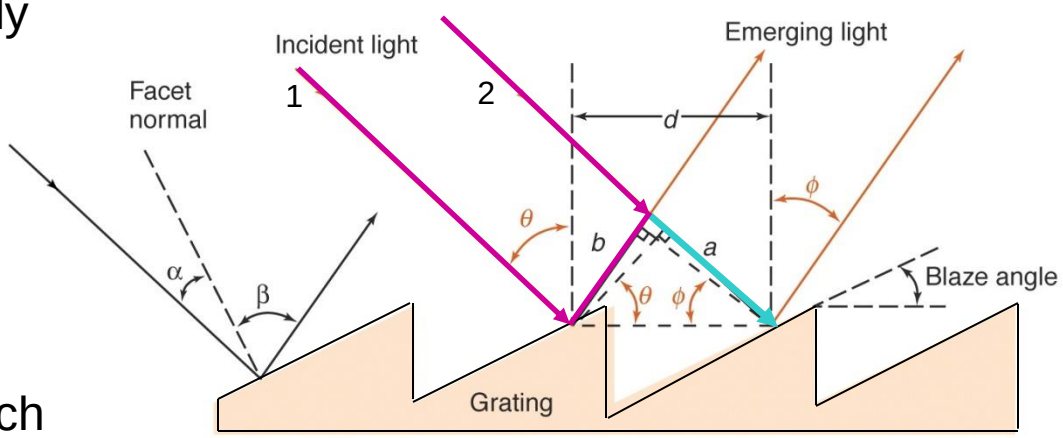
The reflection grating monochromator

Device consists of entrance and exit slits, mirrors, and a grating to disperse the light

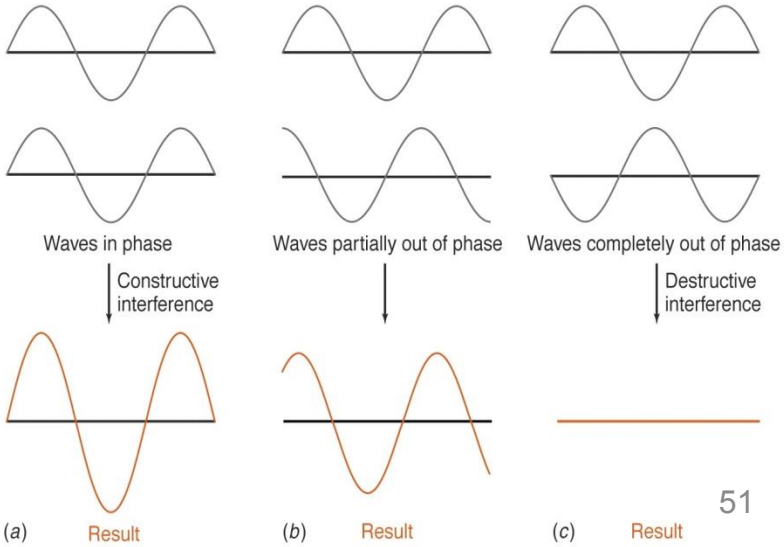
Echellette Reflection Grating



1. The reflection grating is ruled with a series of closely spaced, parallel grooves with repeated distance d.
2. The grating is covered with Al to make it **reflective.**
3. When polychromatic light is reflected from the grating, each groove behaves as **a new point source** of radiation.
4. When adjacent light rays are in phase, they reinforce one another (**constructive interference**).
5. When adjacent light rays are not in phase, they partially or completely canceled one another (**destructive interference**).

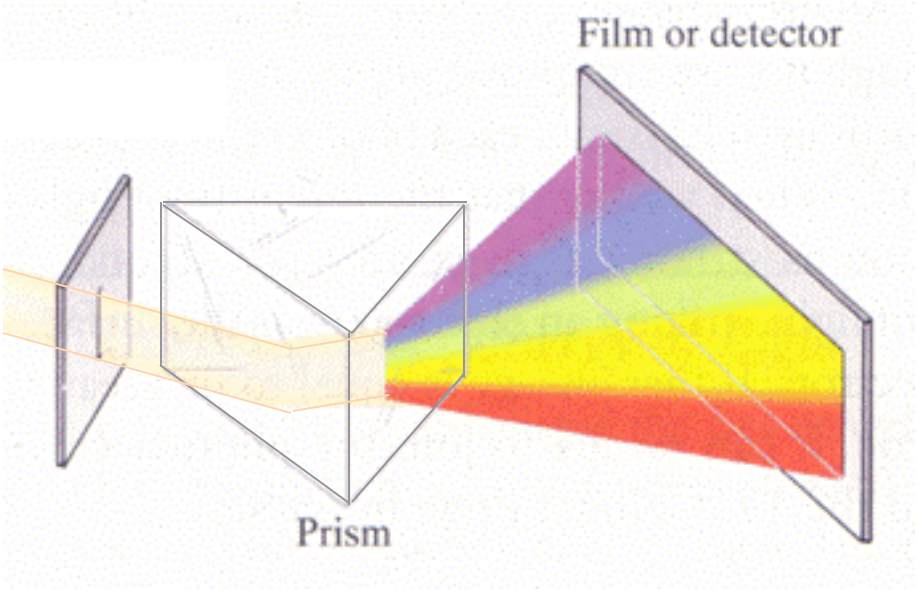


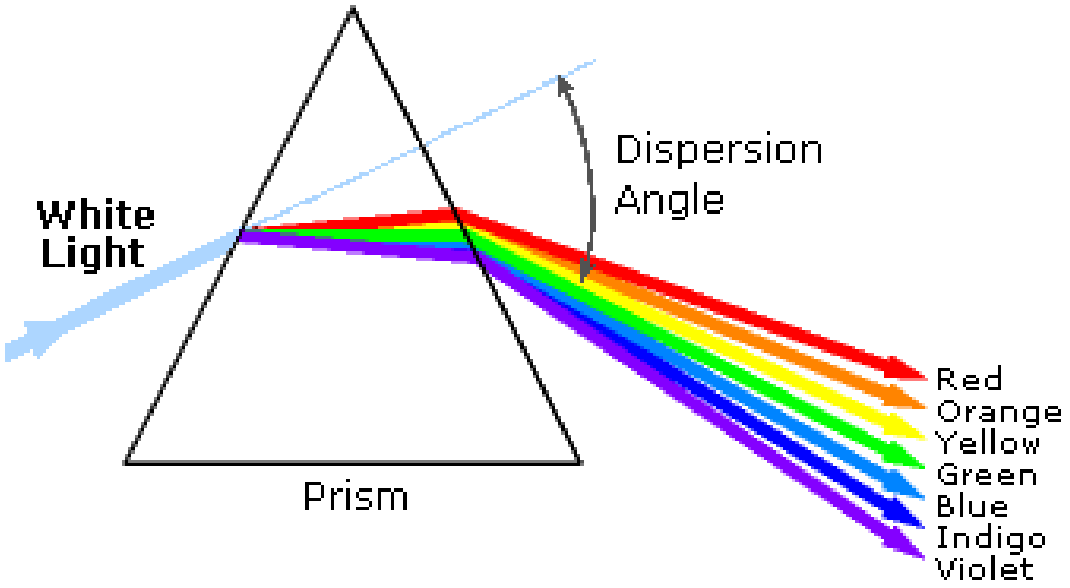
Reflection followed by either constructive or destructive interferences



Prism monochromators

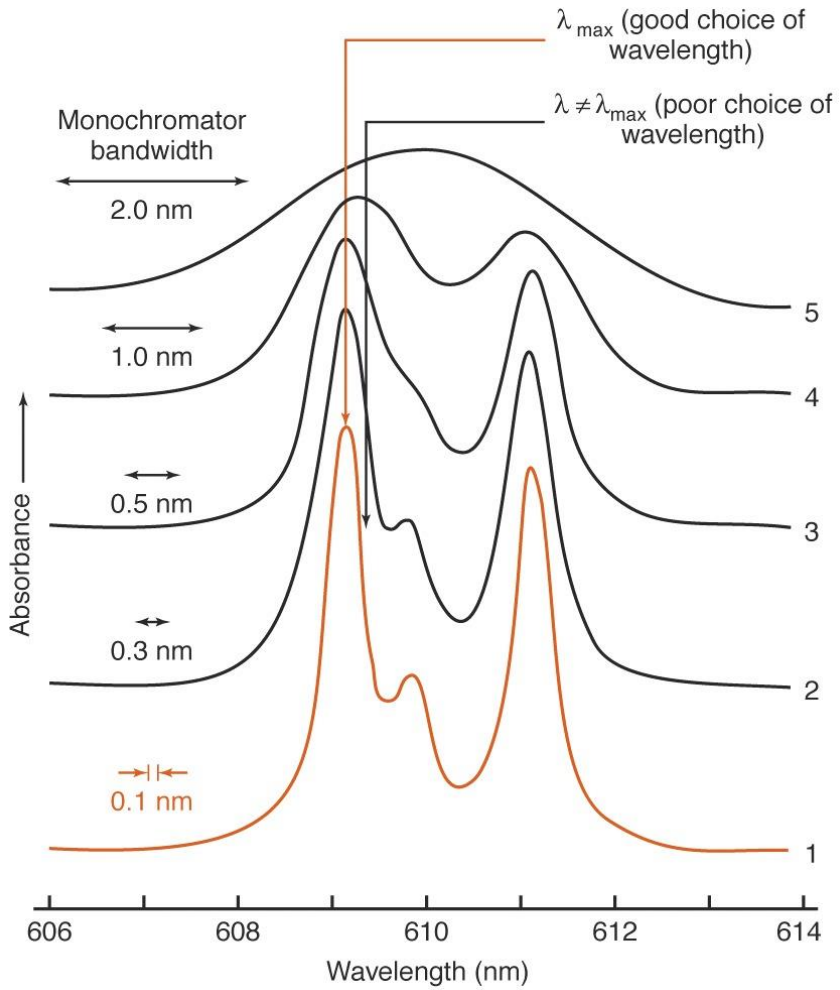
- Dispersion by prism depends on refraction of light which is wavelength dependent
- Violet color with higher energy (shorter wavelength) are diffracted or bent most
- While red light with lower energy (longer wavelength are diffracted or bent least
- As a result, the poly-chromatic white light is dispersed to its individual colors.





What are the advantages and disadvantages of decreasing monochromator slit width?

Bandwidth Choice



The size of the monochromator exit slit determines the width of radiation (**bandwidth**) emitted from the monochromator.

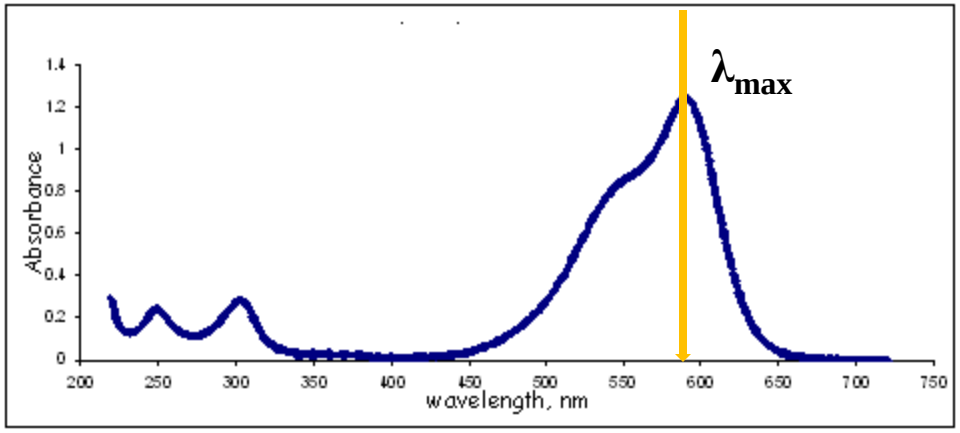
A ***wider*** slit width gives ***higher sensitivity*** because higher radiation intensity passes to the sample but on the other hand, ***narrow*** slit width gives ***better resolution*** for the spectrum.

In general, the choice of slit width to use in an experiment must be made by **compromising** these factors. Still, we can overcome the problem of low sensitivity of the small slit by **increasing the sensitivity of the detector**.

Selection of wavelength

Absorbance measurements are always carried out at **fixed wavelength** (using monochromatic light). When a wavelength is chosen for **quantitative analysis**, three factors should be considered

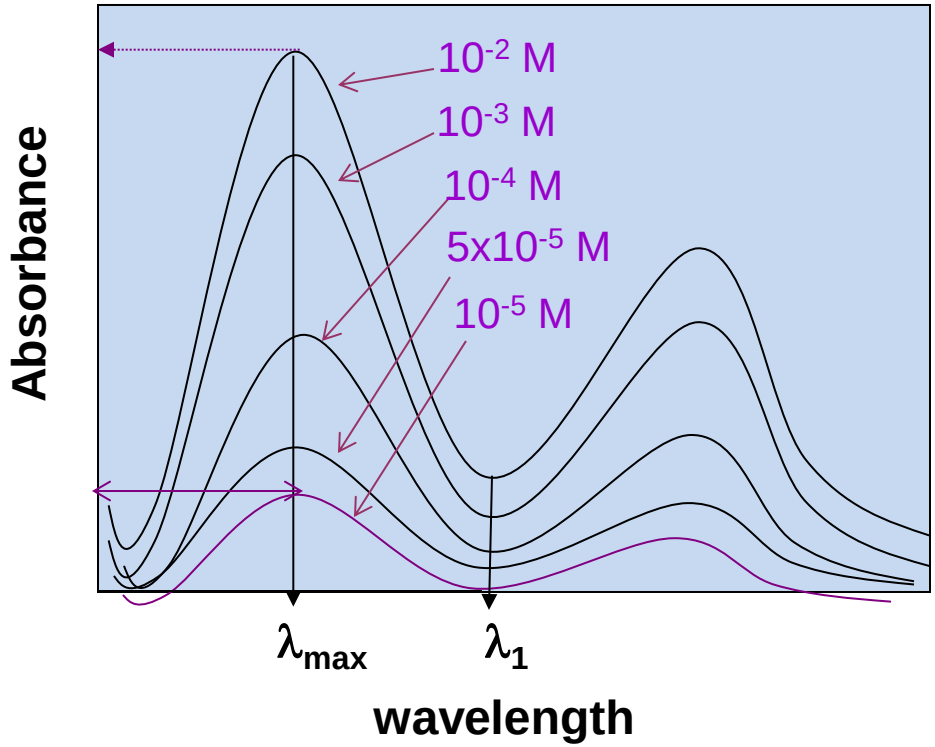
1. **Wavelength should be chosen to give the highest possible sensitivity.**
   This can be achieved by selecting λ_{max} or **in general the wavelengths at which the absorptivity is relatively high.**



λ_{max} - wavelength where maximum absorbance occurs

By performing the analysis at such wavelengths, it will be sure that the lowest sample concentration can be measured with fair accuracy.

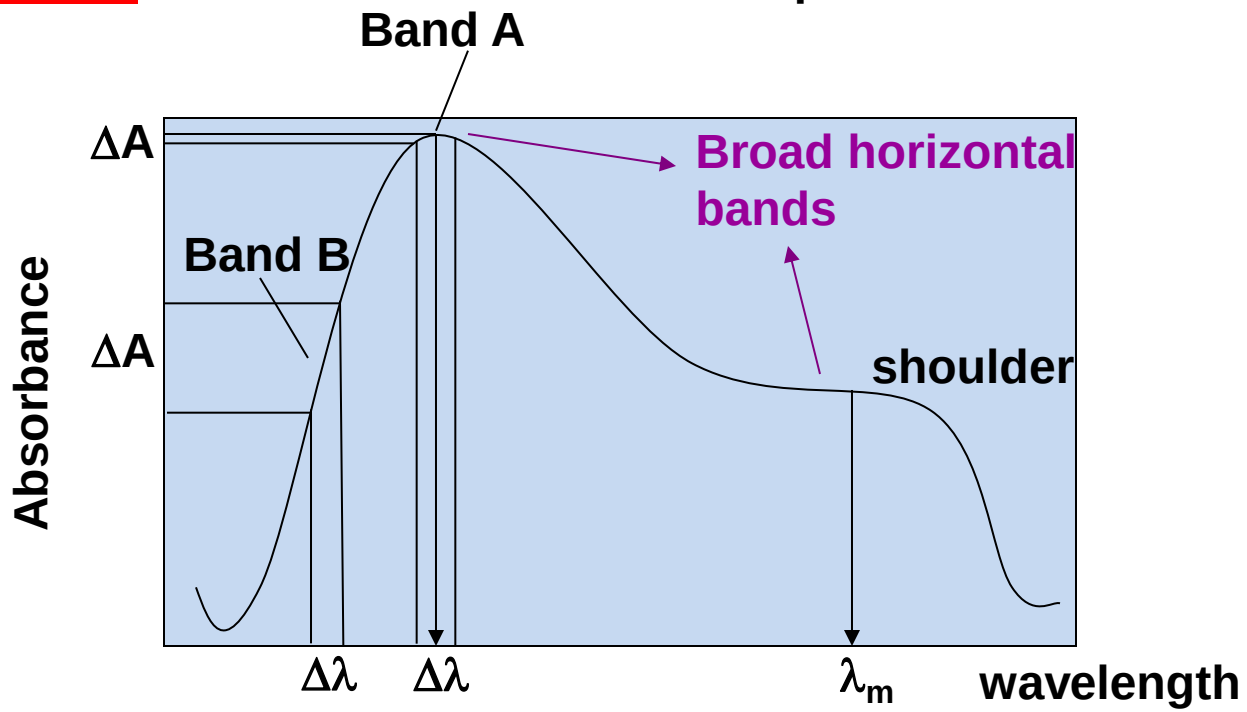
For example, the lowest sample concentration (10^{-5} M) can be measured with good accuracy at λ_{max}, while at other wavelength (λ_1), it may not be detected at all.



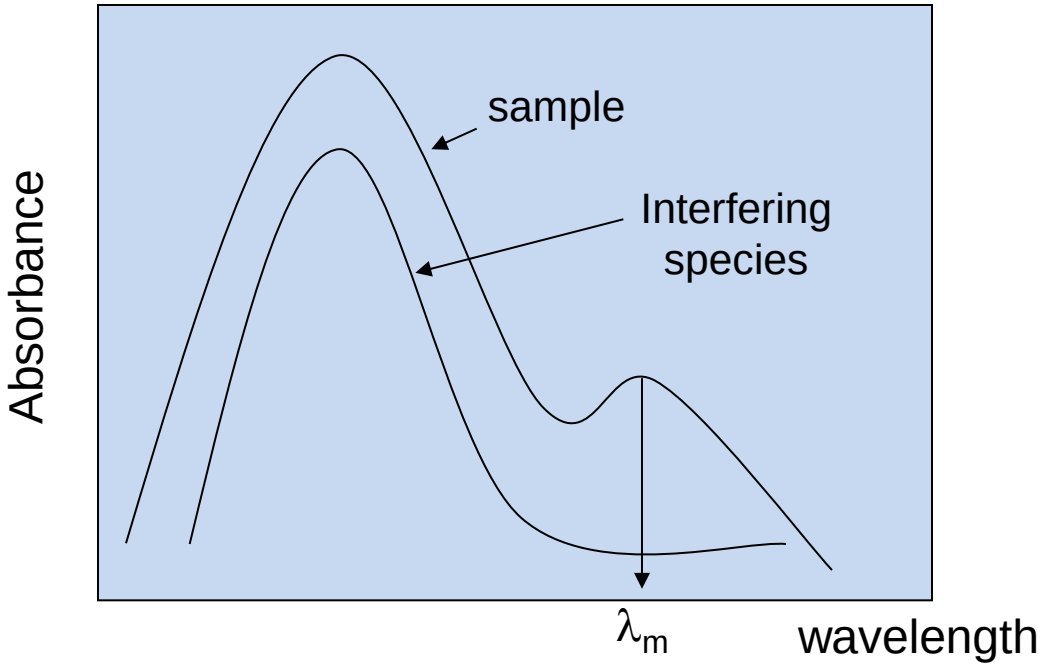
2. **It is preferable to choose the wavelength at which the absorbance will not significantly change if the wavelength is slightly changed, i.e., $\Delta A / \Delta\lambda$ is minimum.**

At a wavelength corresponding to broad horizontal band on the spectrum (band A), the radiation is mainly absorbed to the same extent ($\Delta A / \Delta\lambda$ ~zero).

However on a steep portion of the spectrum (band B), the absorbance will change greatly if the wavelength is changed ($\Delta A / \Delta\lambda$ is large) . Thus on repeating the absorbance measurements, you might get different readings and the precision of the measurements will be poor.

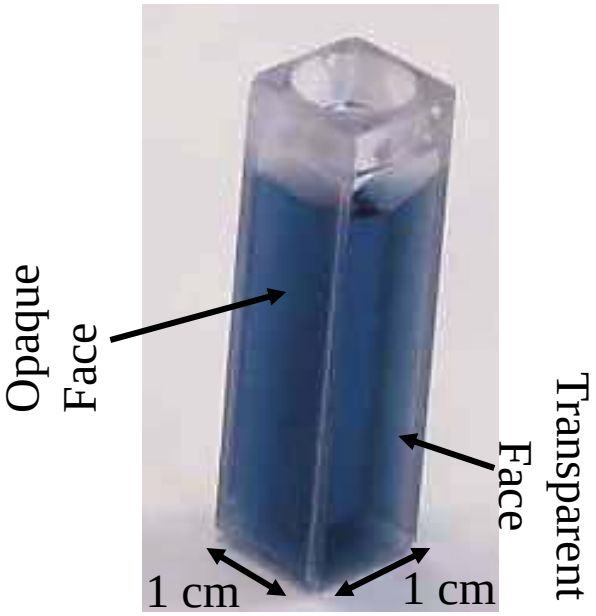


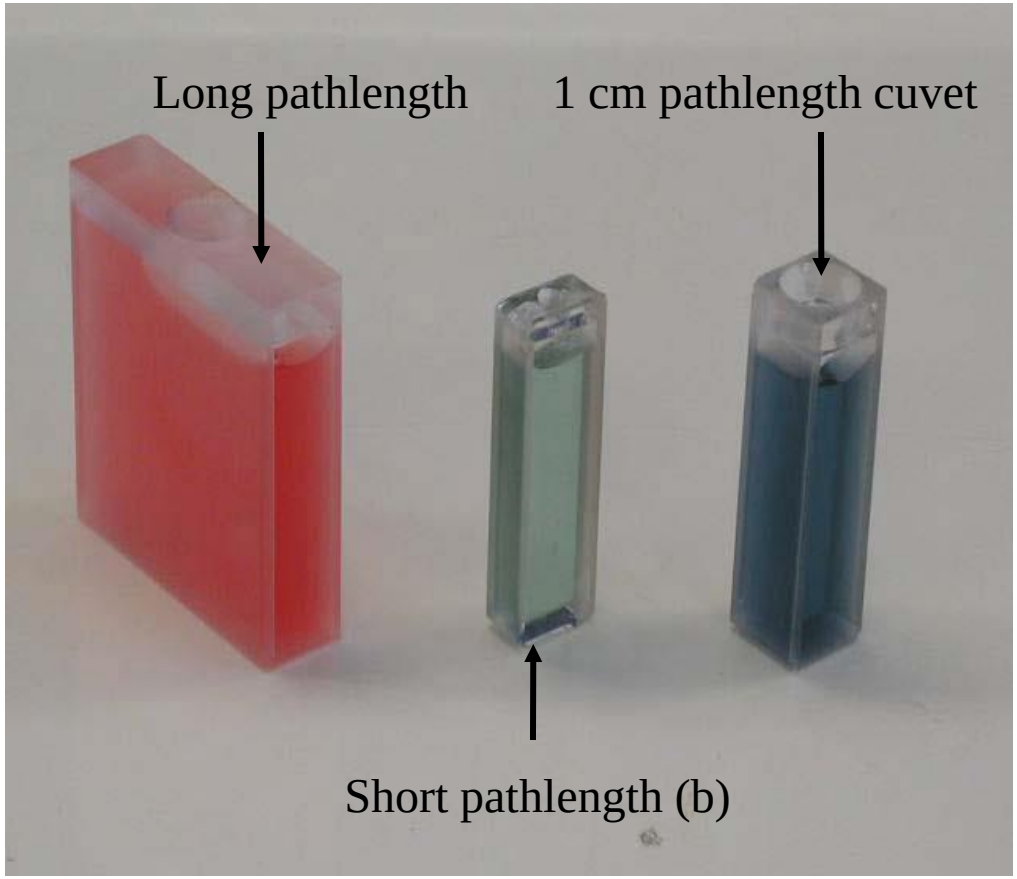
3- If the solution contains more than absorbing species, the wavelength should be chosen, whenever possible, in region at which the other species does not absorb radiation or its absorbance is minimum. By this way, the second species does not interfere in the determination.



Sample compartment (cells)

- For Visible and UV spectroscopy, a liquid sample is usually contained in a cell called a **cuvette**.
- **Glass** is suitable for **visible** but not for UV spectroscopy because it absorbs UV radiation. **Quartz** can be used in **UV** as well as in visible spectroscopy



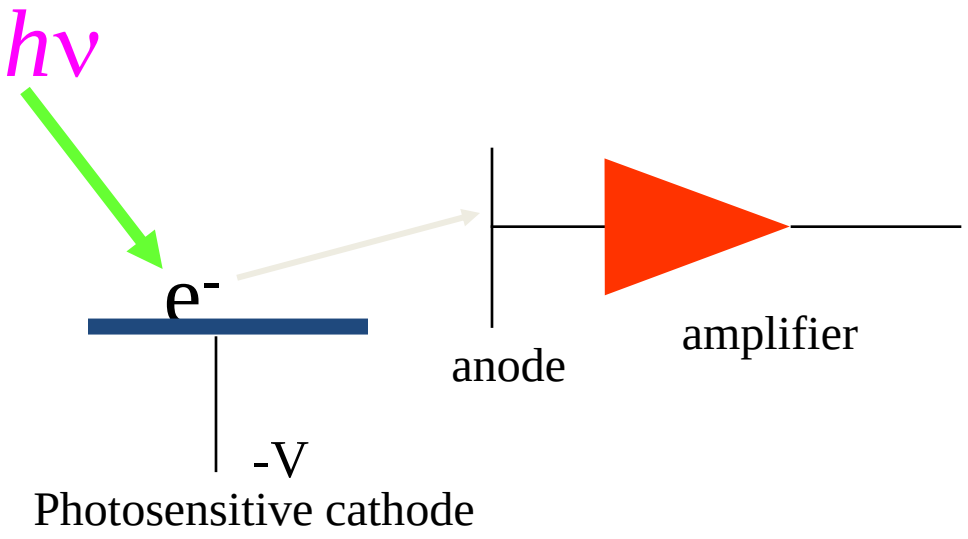


Detectors

- The detectors are devices that convert radiant energy into electrical signal.
- A Detector should be sensitive, and has a fast response over a considerable range of wavelengths.
- In addition, the electrical signal produced by the detector must be directly proportional to the transmitted intensity (linear response).

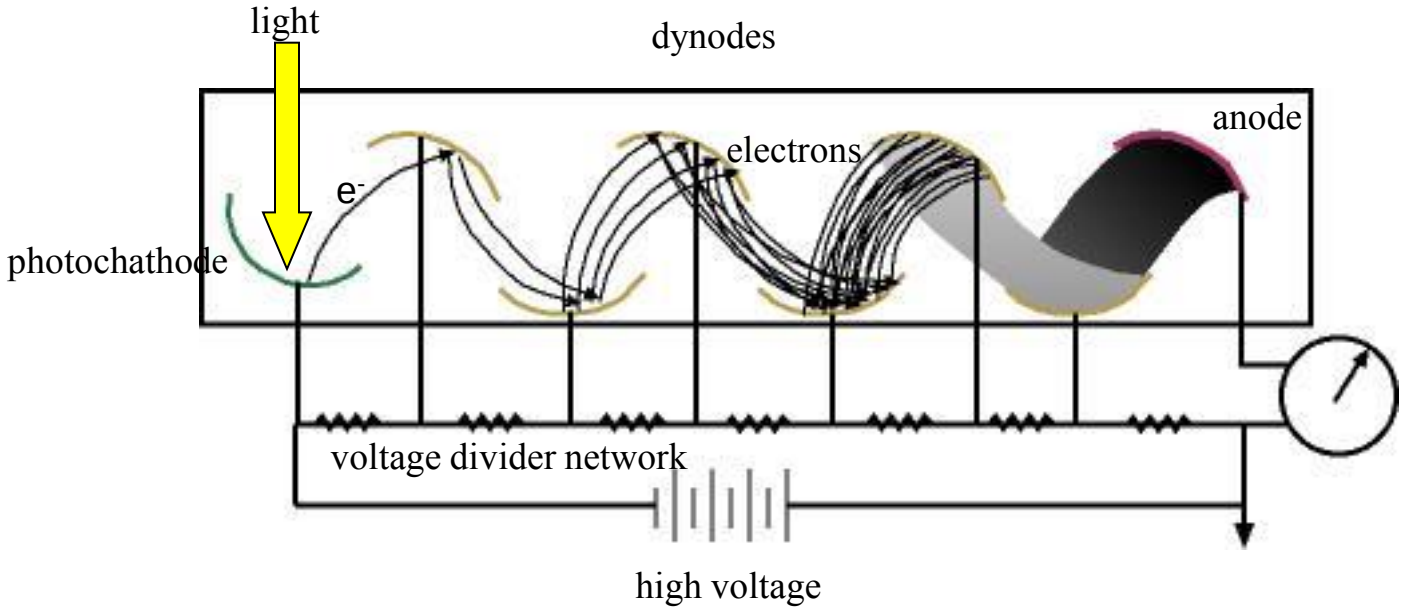
i- Phototube

- Phototube emits electrons from a **photosensitive, negatively charged cathode** when struck by visible or UV radiation
- The electrons flow through vacuum to **an anode** to produce current which is proportional to radiation intensity.

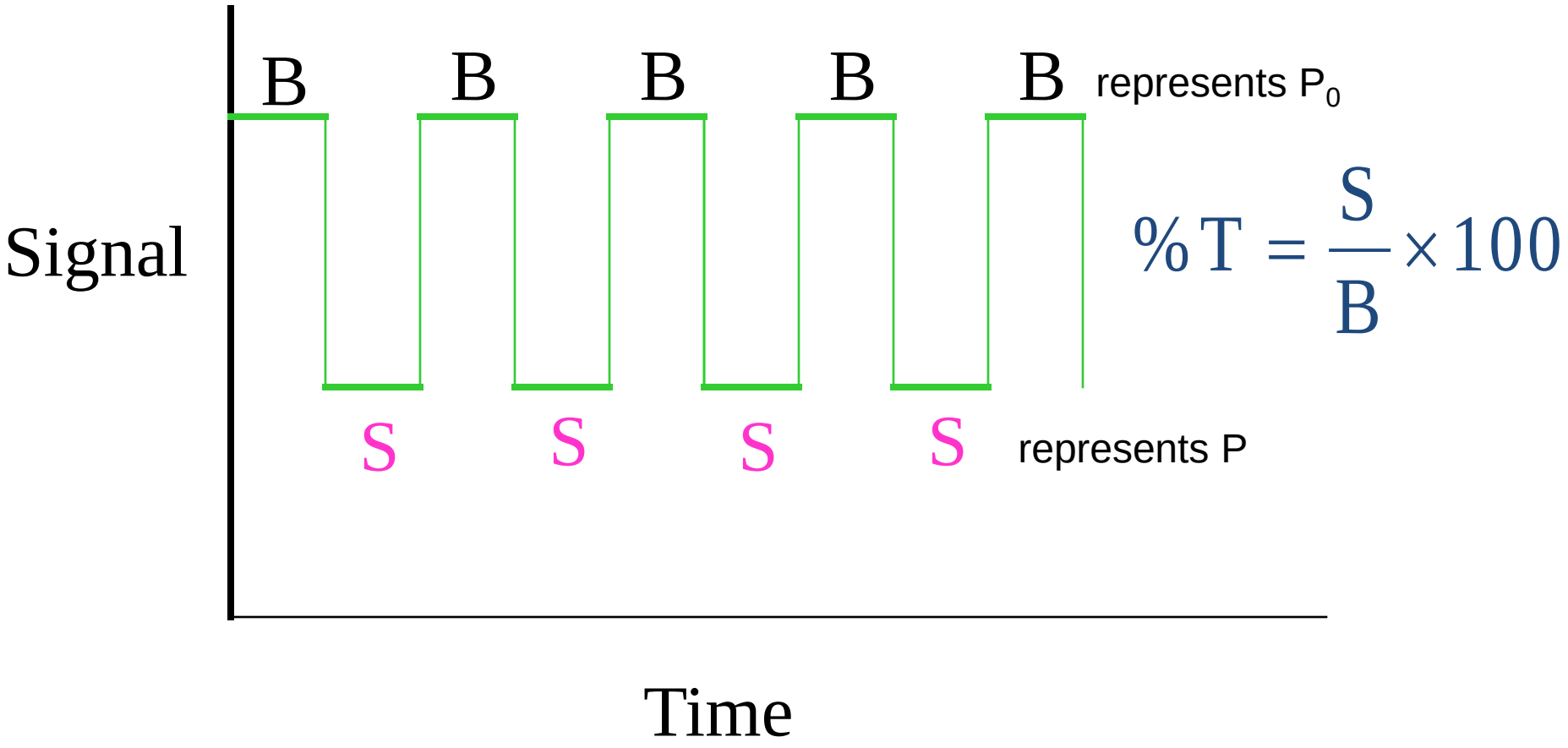


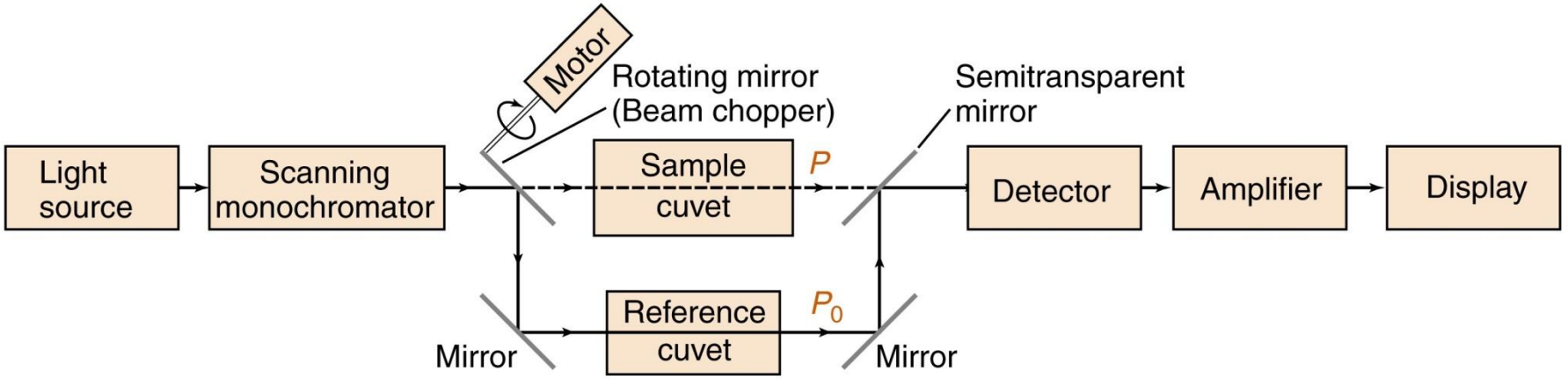
Photomultiplier tube

- It is a very sensitive device in which electrons emitted from the photosensitive cathode strike a second surface called **dynode** which is positive with respect to the original cathode.
- Electrons are thus accelerated and can knock out more than one electrons from the dynode.
- If the above process is repeated several times, so more than 10^6 electrons are finally collected for each photon striking the first cathode.



Double Beam Spectrophotometer





Schematic diagram of a double beam scanning spectrophotometer

* In double beam arrangement, the light alternately passes through **the sample and reference** (blank), directed by rotating half-sector mirror (chopper) into and out of the light path.

* When light passes through the sample, the detector measures the **P**. When the chopper diverts the beam through the blank solution, the detector measures **P_0.**

* The beam is chopped several times per second and the electronic circuit automatically compares **P and P_0** to calculate absorbance and Transmittance.

Advantages of double beam instruments over single beam instruments

Single beam spectrophotometer is inconvenient because

1. The sample and blank must be placed alternately in the light path.
2. For measurements at multiple wavelengths, the blank must be run at each wavelength.

In double beam instruments

1. The absorption in the sample is automatically corrected for the absorption occurring in the blank, since the readout of the instrument is log the difference between the sample beam and the blank beam.
2. Automatic correction for changes of the source intensity and changes in the detector response with time or wavelength because the two beams are compared and measured at the same time.
3. Automatic scanning and continuous recording of spectrum (absorbance versus wavelength).

Applications of Ultraviolet/Visible Molecular Absorption Spectrophotometry

- Molecular spectroscopy based upon UV-Vis radiation is used for **identification and estimation** of inorganic, organic and biomedical species.
- Molecular UV-Vis absorption spectrophotometry is employed primarily for **quantitative analysis.**
- UV/Vis spectrophotometry is probably more widely used in **chemical and clinical** laboratories throughout the world than any other single method.

The important characteristics of Spectrophotometric methods

1. *Wide applicability* to both organic and inorganic systems
2. *High sensitivity* of **10^{-6}-10^{-4}** M
3. *Moderate to* **high selectivity.**
4. ***Good accuracy*** the relative error encountered in concentration lie in the range from **1% to 3%**
5. **Ease and convenience** *of data acquisition*

Resources and references

- Textbook: Principles of instrumental analysis, Skoog et al., 5th edition, chapter 7, 13.
- Quantitative chemical analysis, Daniel C. Harris, 6th edition , chapter 20.
- Lecture slides partially adopted from Dr. Raafat Aly slides.
- Useful links

http://www.youtube.com/watch?v=pxC6F7bK8CU&feature=player_detailpage

http://bio-animations.blogspot.com/2008/04/double-beam-uvvis-spectrophotometer.html

Spectral properties of biological molecules

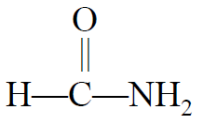
- Much more complex than FA
- Major constrains – solvents and solvation
- In water experiments at $\lambda > 170$nm, water is strongly polar, electronic absorption bands are broader than in most other solvents (at various orientations and distances), problem of temperature (1 – 100^0C)

Protein chromophores

peptide bond itself | aminoacid side chains | any prosthetic groups

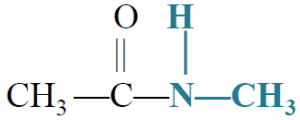
MODEL

formamide



methanamide (IUPAC)
formamide (common)

N-methylacetamide



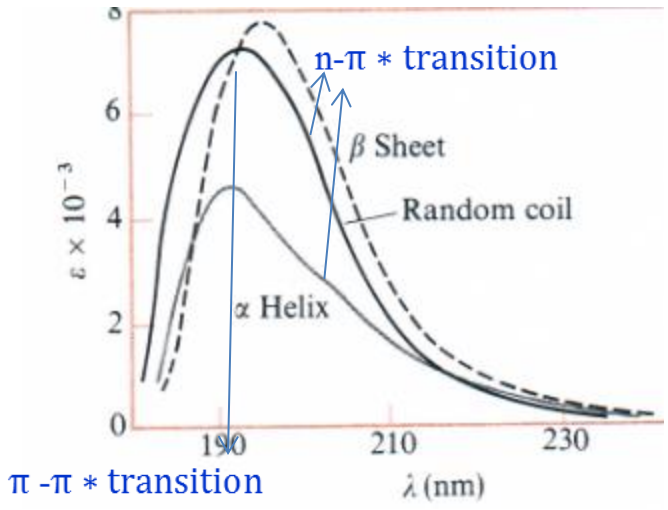
N-methylethanamide (IUPAC)
N-methylacetamide (common)

π electrones are delocalized over C,O,N

n-π* transition – the lowest energy of electronic transition is symmetry – forbidden

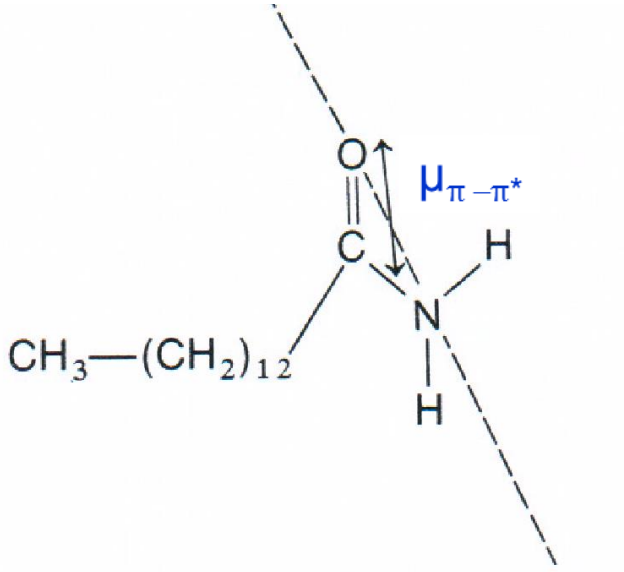
210-220 nm, ε_{max} ~ 100

UV spectra of poly-L-lysine in aqueous solutions



Ultraviolet absorption spectrum of poly-L-lysine in aqueous solution: random coil, pH 6.0, 25°C; α helix, pH 10.8, 25°C; β sheet pH 10.8, 52°C. [After K. Rosenheck and P. Doty, *Proc. Natl. Acad. Sci. USA* 47:1775 (1961).]

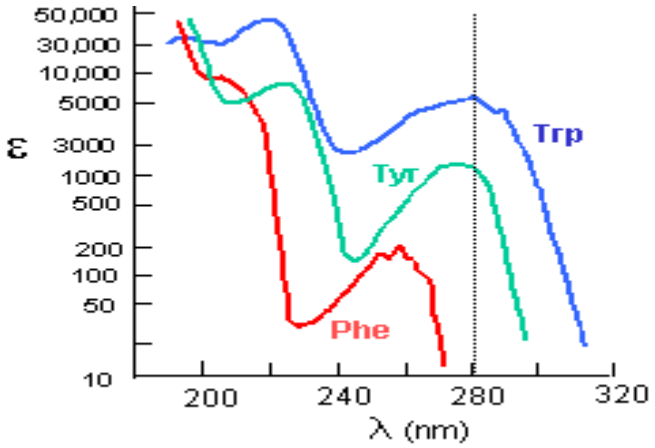
In FA π -π ∗ transition is polarized along the C − O axis, in peptide does not lie along any particular bond

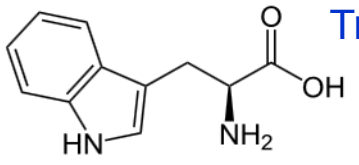


UV spectra of aromatic amino acid

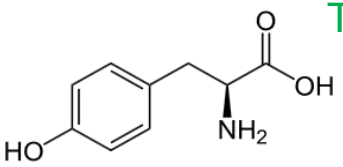
AA – in the same spectral region – the strong peptide absorption (π-π*)

AAA – in pH 7 (π-π*) symmetry

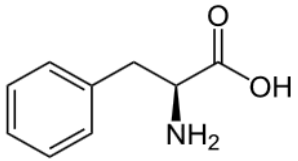




Tryptophane

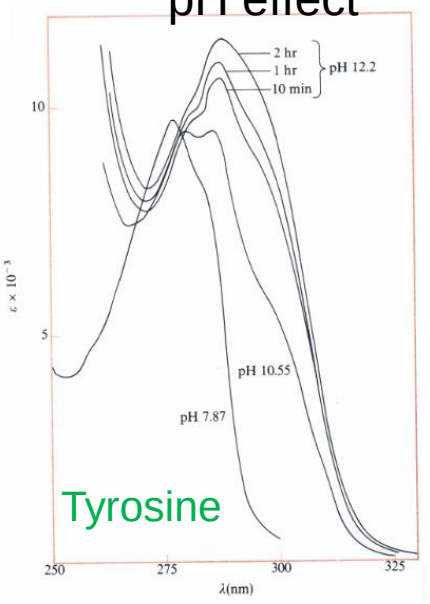


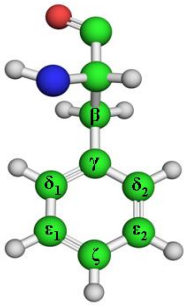
Tyrosine



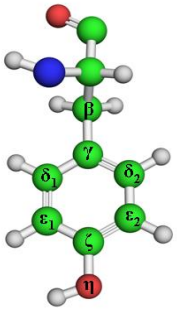
Phenylalanine

pH effect

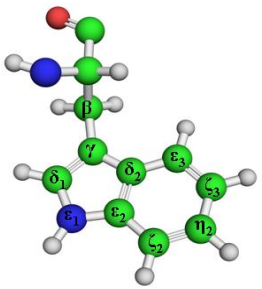




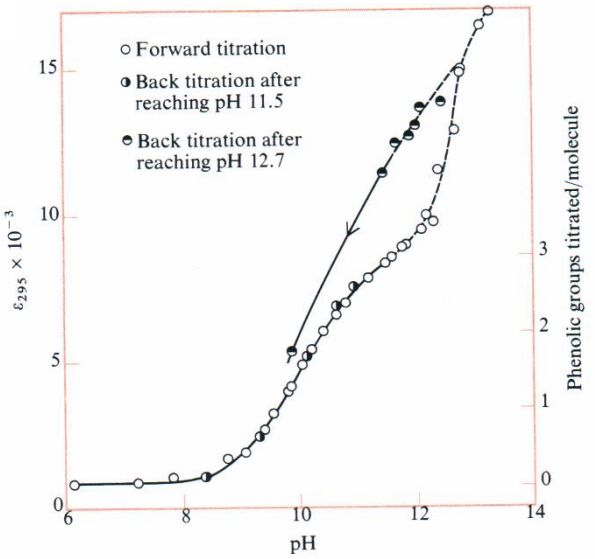
Phenylalanine
(Phe, F)



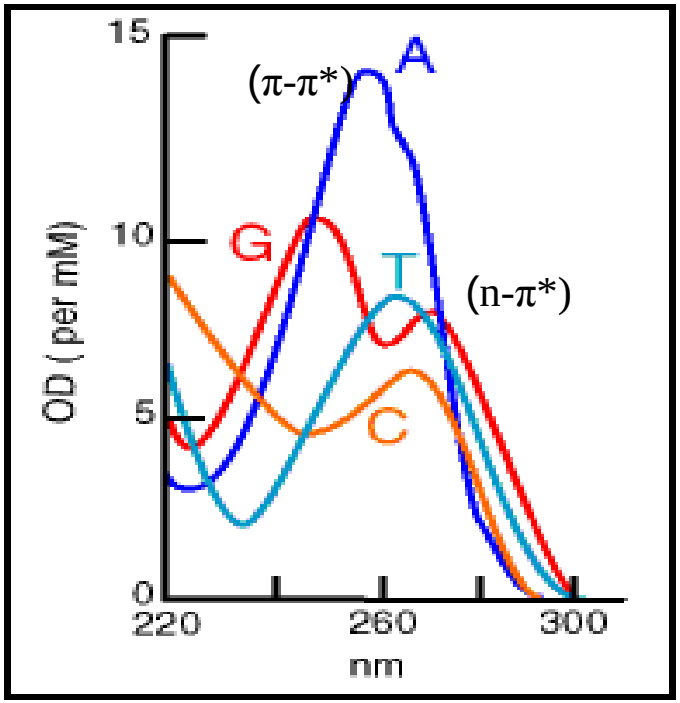
Tyrosine
(Tyr, Y)

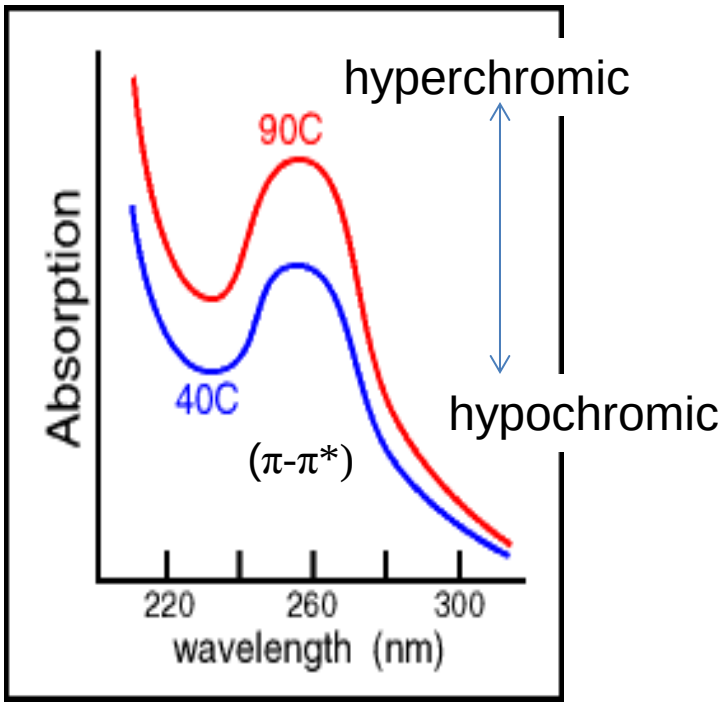


Tryptophan
(Trp, W)



UV spectra of DNA and its bases





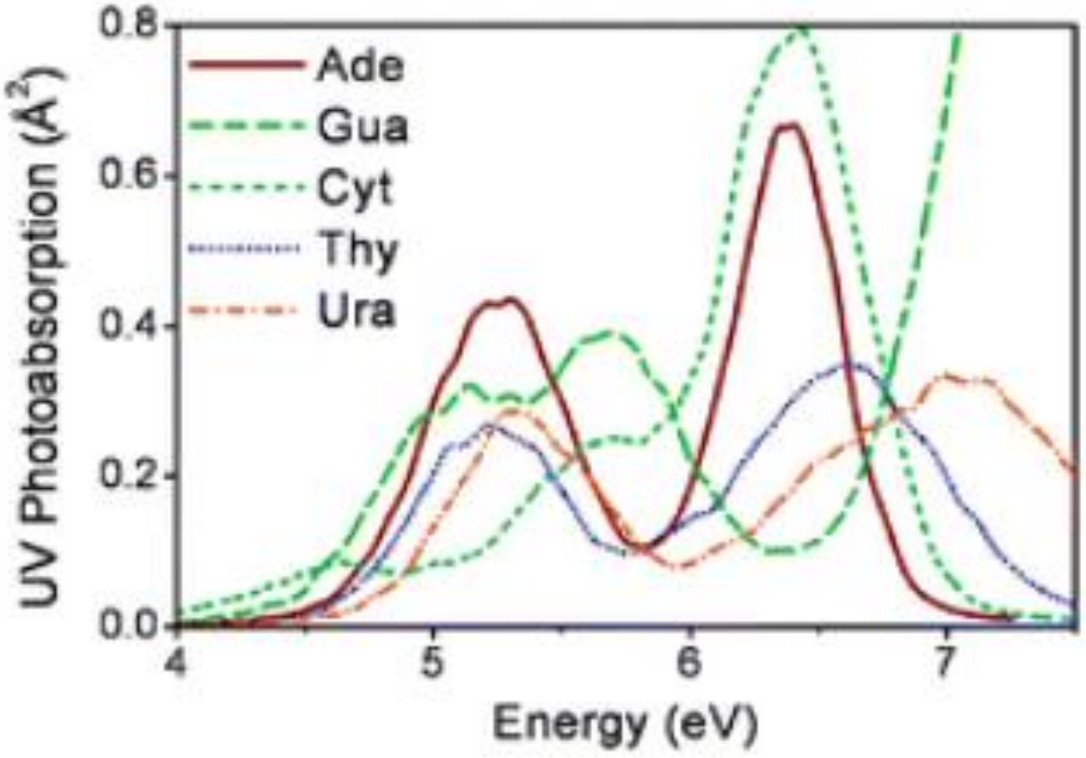
A pure DNA solution appears transparent to the eye, and absorption doesn't become measurable until 320 nm. Moving further into the u.v. region, there is a peak at about 260 nm, followed by a dip between 220 and 230, and then the solution becomes essentially opaque in the far u.v.

When a DNA helix is denatured to become single strands, e.g. by heating, the absorbance is increased about 30 percent. This increase, called the hyperchromic effect, reveals the interaction between the electronic dipoles in the stacked bases of the native helix.

Electronic state of nucleobases is more complex than chromophores of peptides

- low symmetry
- many nonbonded electrons
- several different transition (π-π*) (n-π*)

The UV absorption of nucleobases: semi-classical *ab initio* spectra simulations

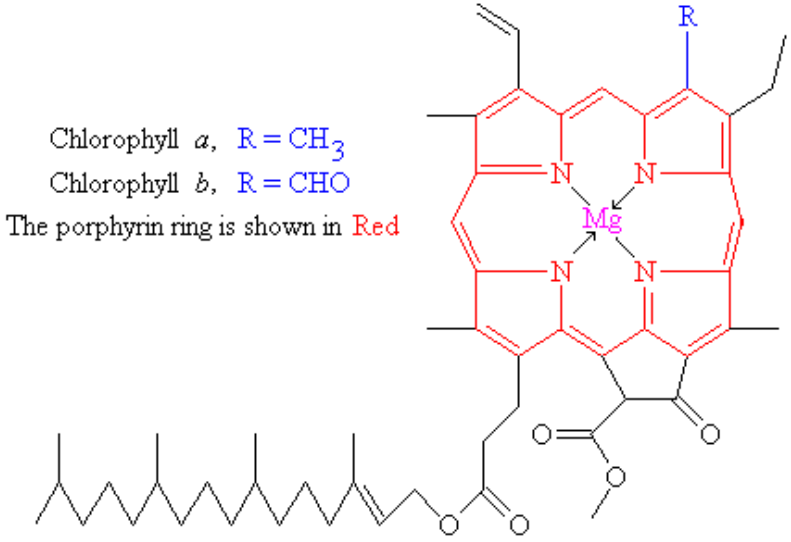


Physical Chemistry Chemical Physics

Effect prosthetic groups

Polypeptide chain – prosthetic group (apoprotein)

- local environment
- oxidation-reduction
- 200 – 300 nm



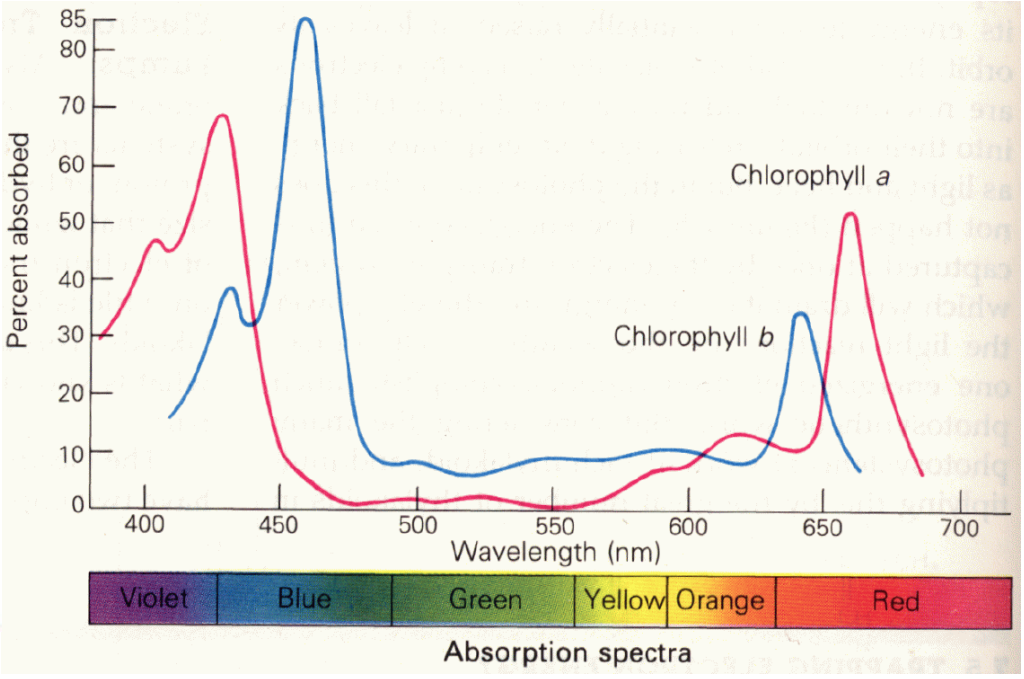
Chlorophyll *a*, R = CH_3
Chlorophyll *b*, R = CHO
The porphyrin ring is shown in Red

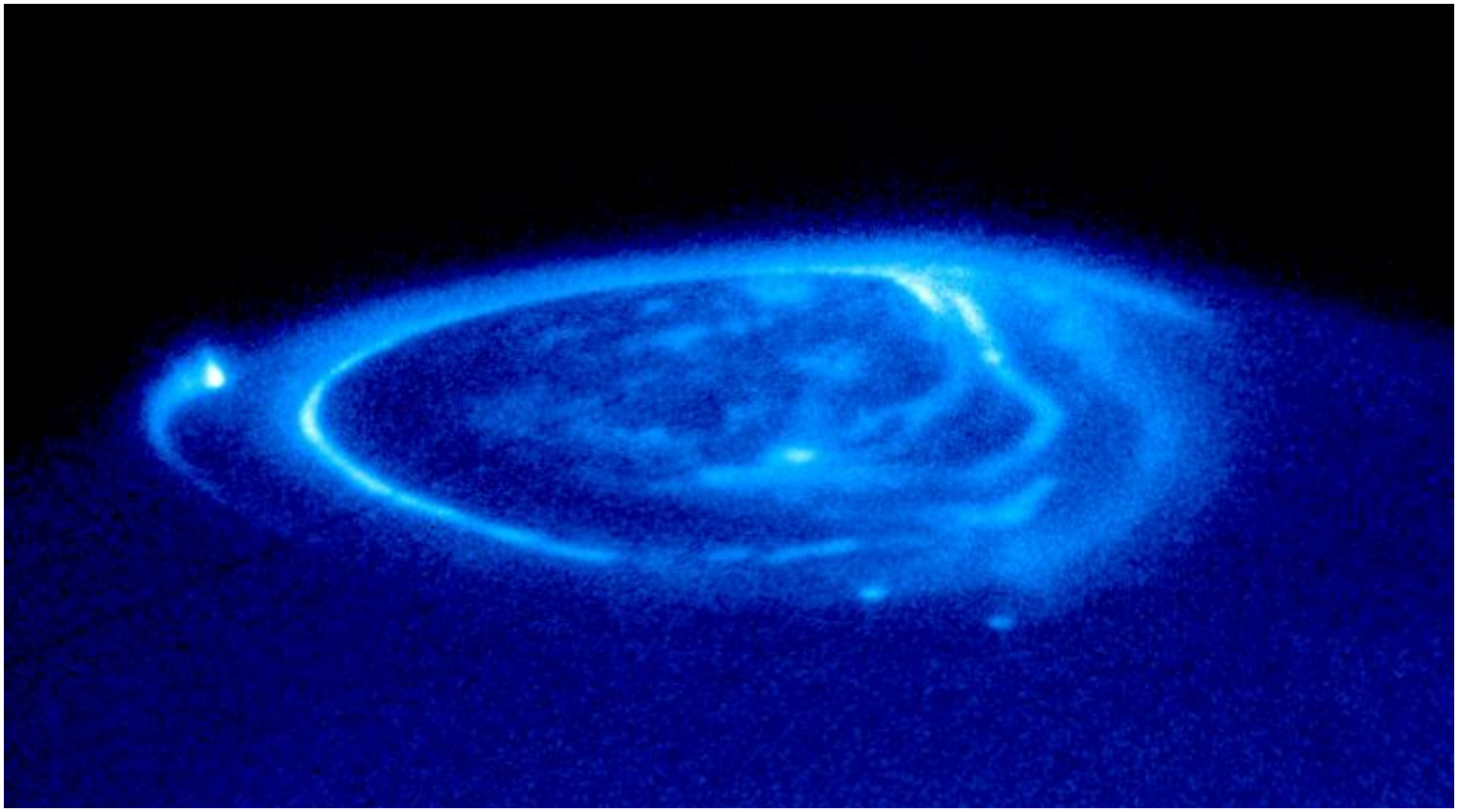
- Prosthetic group must have a high enough molar extinction coefficient to be detectable at typical protein concentrations
- To avoid the formation of intermolecular aggregates

Spectroscopic properties of proteins containing prosthetic groups

Protein	Prosthetic group	Longest-wavelength absorption band λ_{max} (nm)	Longest-wavelength absorption band ε_{max} ($\times 10^{-4}$)	Second-longest absorption band λ_{max} (nm)	Second-longest absorption band ε_{max} ($\times 10^{-4}$)
Amino acid oxidase, rat kidney	FMN	455	1.27	358	1.07
Azurin, *P. fluorescens*	Cu^{II}	781	0.32	625	0.35
Ceruloplasmin, human	8 Coppers (3 distinct classes)	794	2.2	610	1.13
Cytochrome *c*, reduced, human	Fe^{II}-heme	550	2.77	—	—
Ferredoxin, *Scenedesmus*	(2 Fe^{III}, 2 sulfide) cluster	421	0.98	330	1.33
Flavodoxin, *C. pasteurianium*	FMN	443	0.91	372	0.79
Monoamine oxidase, bovine kidney	Flavins plus Cu	455	4.7	—	—
Pyruvic dehydrogenase, *E. coli*	FAD	460	1.27	438	1.46
Rhodopsin, bovine	Retinal-Lys	498	4.2	350	1.1
Reubredoxin, *M. aerogenes*	(Fe^{III}, 4 Cys) tetrahedron	570	0.35	490	0.76
Threonine deaminase, *E. coli*	4 Pyridoxal phosphates	415	2.6	—	—
Xanthine oxidase	Fe, Mo	550	2.2	—	—

Absorption spectra of chlorophylls





Snímek polární záře na Jupiteru, jak ji v ultrafialovém oboru spektra zaznamenal Hubbleův vesmírný dalekohled

Podle moderních modelů evoluce je vznik a evoluce prvotních proteinů a enzymů schopných reprodukce připisován právě existenci ultrafialového záření.