Part (1)
Fundamentals of Spectrophotometry
UV-VIS-IR
Luminescence
Atomic Spectroscopy

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Fundamentals of Spectrophotometry

Reminder

Properties of Light

Absorption of Light

The Spectrophotometer

Beer’s Law in Chemical Analysis

What happens when a molecule absorbs light?

Luminescence

Analytical Chemistry

- Identification,
- Determination of Ingredient substances,
- Stability study,
- Bioequivalence study,
- Bioavailability study,
- Base of most supplemented sciences of pharmacy,
- Research and development of pharmacy,
- Drug industry,
- Drug delivery, (A new science branch, Pharmacy, Chemistry, Biology, etc.),

Analytical Chemistry

- Qualitative analysis
- Quantitative analysis

- Aspirin Form (Stability)
- Solubility

Reminder

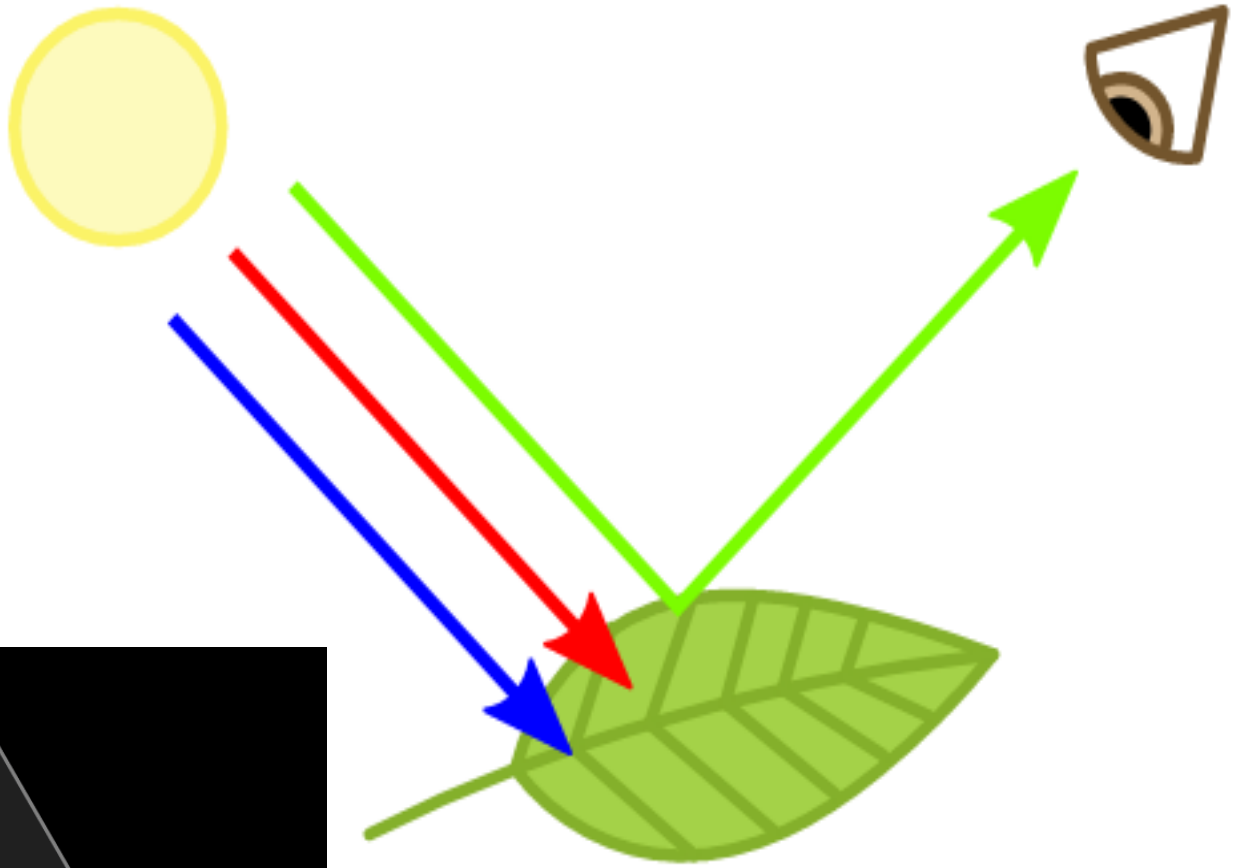
- Prefixes of SI units

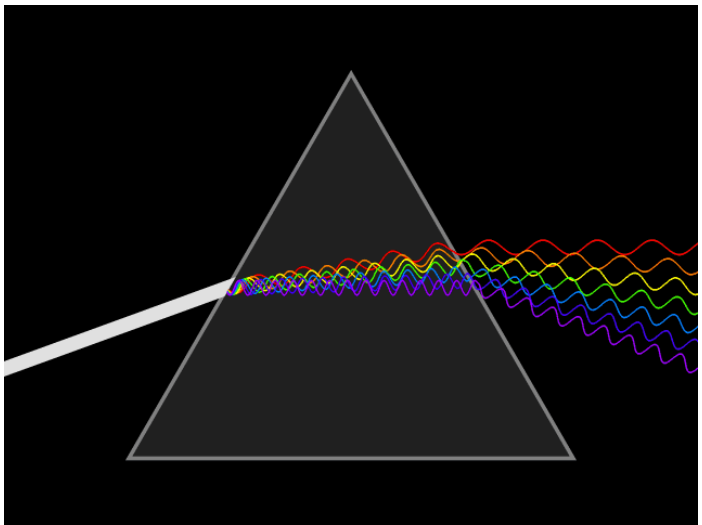
pico	nano	micro	milli	centi	kilo	mega
p	**n**	**μ**	**m**	**c**	**k**	**M**
10^{-12}	10^{-9}	10^{-6}	10^{-3}	10^{-2}	10^{3}	10^{6}

- Example:

$$\frac{10^{-9}\,\mathrm{m}}{\mathrm{nm}} \text{ or } \frac{1\,\mathrm{m}}{10^{9}\,\mathrm{nm}}$$

Reminder





Reminder

Concentration units

1. Mass Percent,
2. Volume Percent,
3. Mole Fraction,
4. Molarity,
5. Molality,
6. Normality,
7. Parts per Million, Parts per Billion, Parts per Trillion.

Reminder: Calibration Methods

- **Instrument calibration**

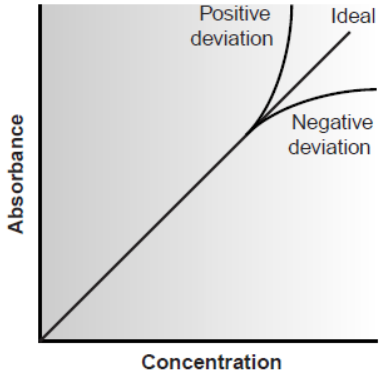
- A new instrument,
- After reparation.
- After a specified time period, Operating hours,
- After/before a critical measurement,
- After an event, i.e., shock, accident, questionable observations, instrument manufacturer recommendation.

- **Calibration Curve: Conc.---Response**

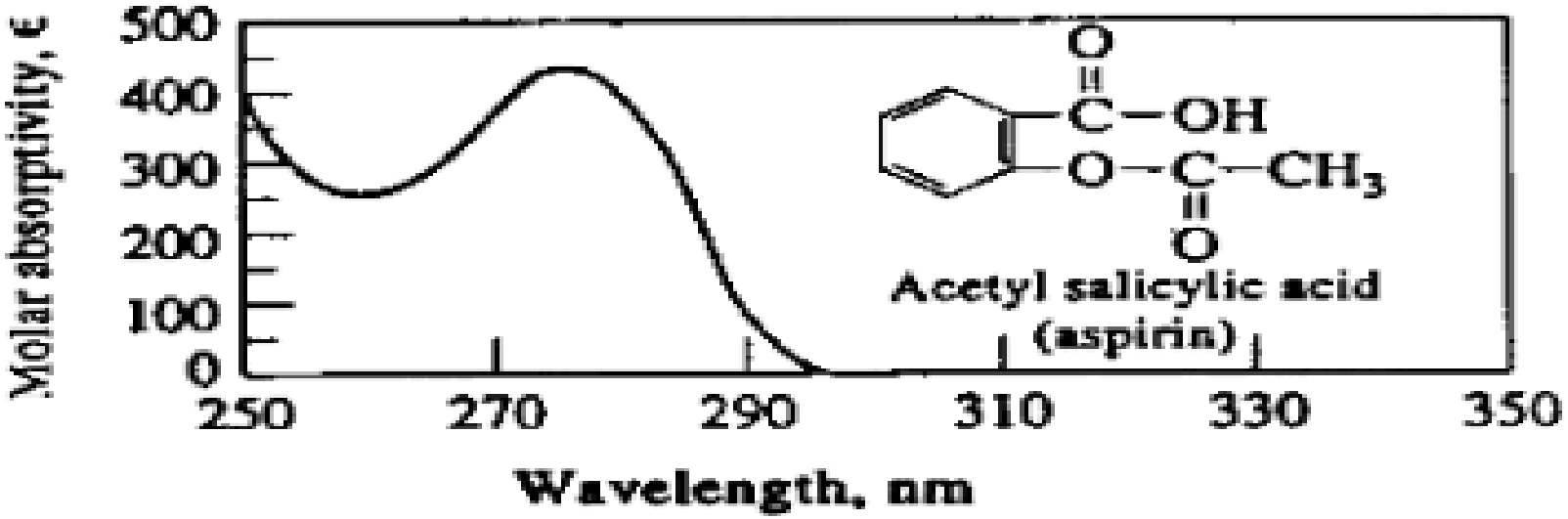
✓ External Standard

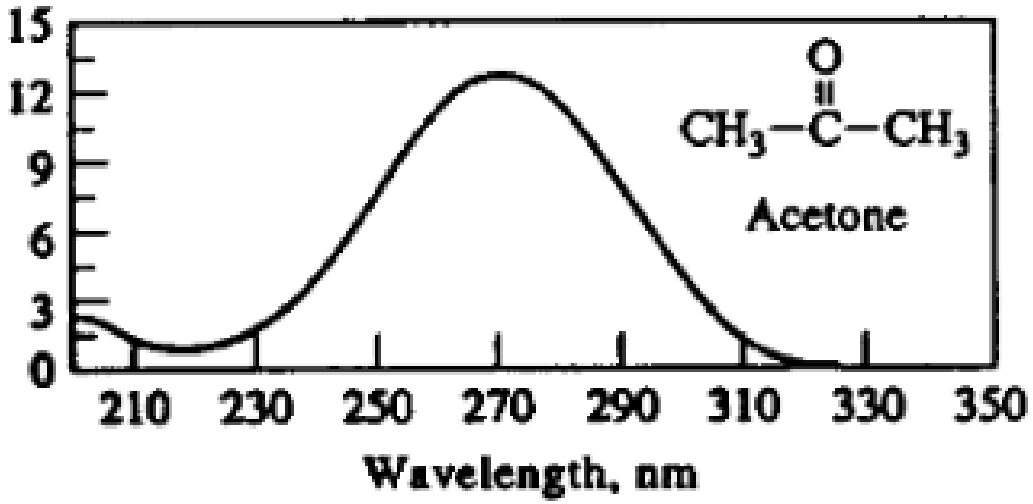
✓ The Standard Addition Method

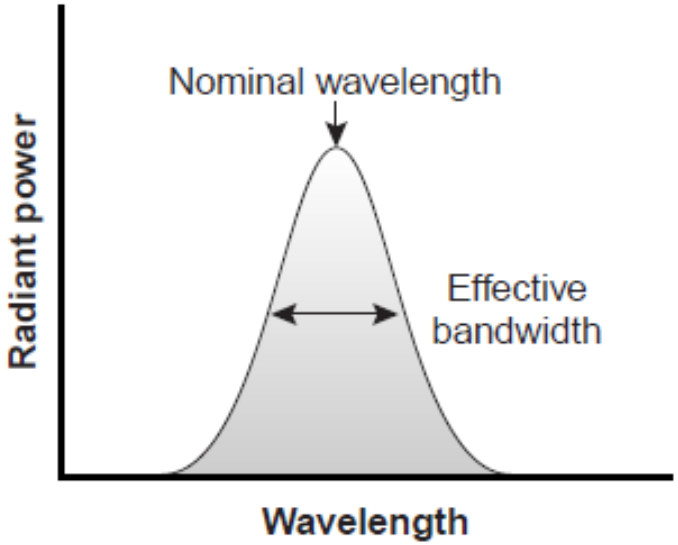
- Sample treatment: Recovery
- Matrix effect: Calibration correction
- Response factor: Calibration correction



Reminder: UV Spectra







Chromophore and Auxochromes

CHROMOPHORE: any group which exhibit absorption of electromagnetic radiation in a visible or ultra-visible region.

Example: ethylenes (C═C), acetylenes(C≡C), -carbonyls (C═O), nitriles (C≡N), azo compounds (−N=N−) and nitro compounds (-NO_2)

Chromophore and Auxochromes

AUXOCHROMES: a group which itself does not act as a chromophore, but when attached to a chromophore, it shifts the adsorption towards longer wavelength along with an increase in the intensity of absorption.

(-OH), (-NH_2), (-OR), (-NHR), and (–NR_2).

Example: When the auxochrome (–NH_2) group is attached to benzene ring. Its absorption change from λ_{max} 225 to λ_{max} 280.

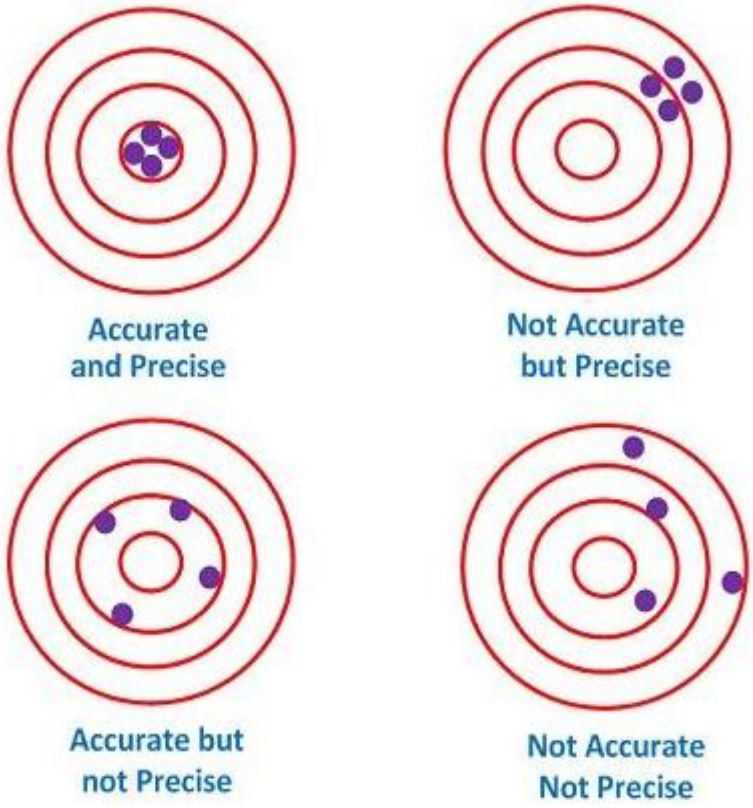
Reminder

Accuracy: the ability of the instrument to measure the accurate value.

Precision: the variability between repeated measurements: Repeatability and Reproducibility.

Sensitivity: the rate of change of the measured response with change in the concentration of analyte.

Selectivity: the accuracy of its measurement in the presence of interferences, i.e., impurities or matrix components.



Reminder: Electronic spin states of molecules

The ground electronic state (a)

In the lowest energy or ground state, the spins are always paired, and the state is a

singlet state.

Excited electronic states:

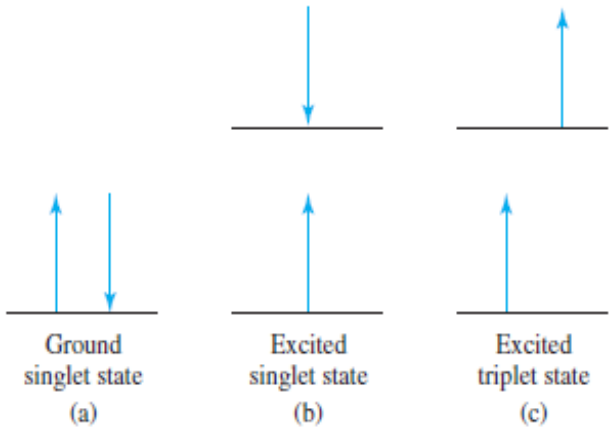
Excited singlet state (b)

The spins remain paired in the excited state,

Excited triplet state (C)

The spins become unpaired,

Doublet???

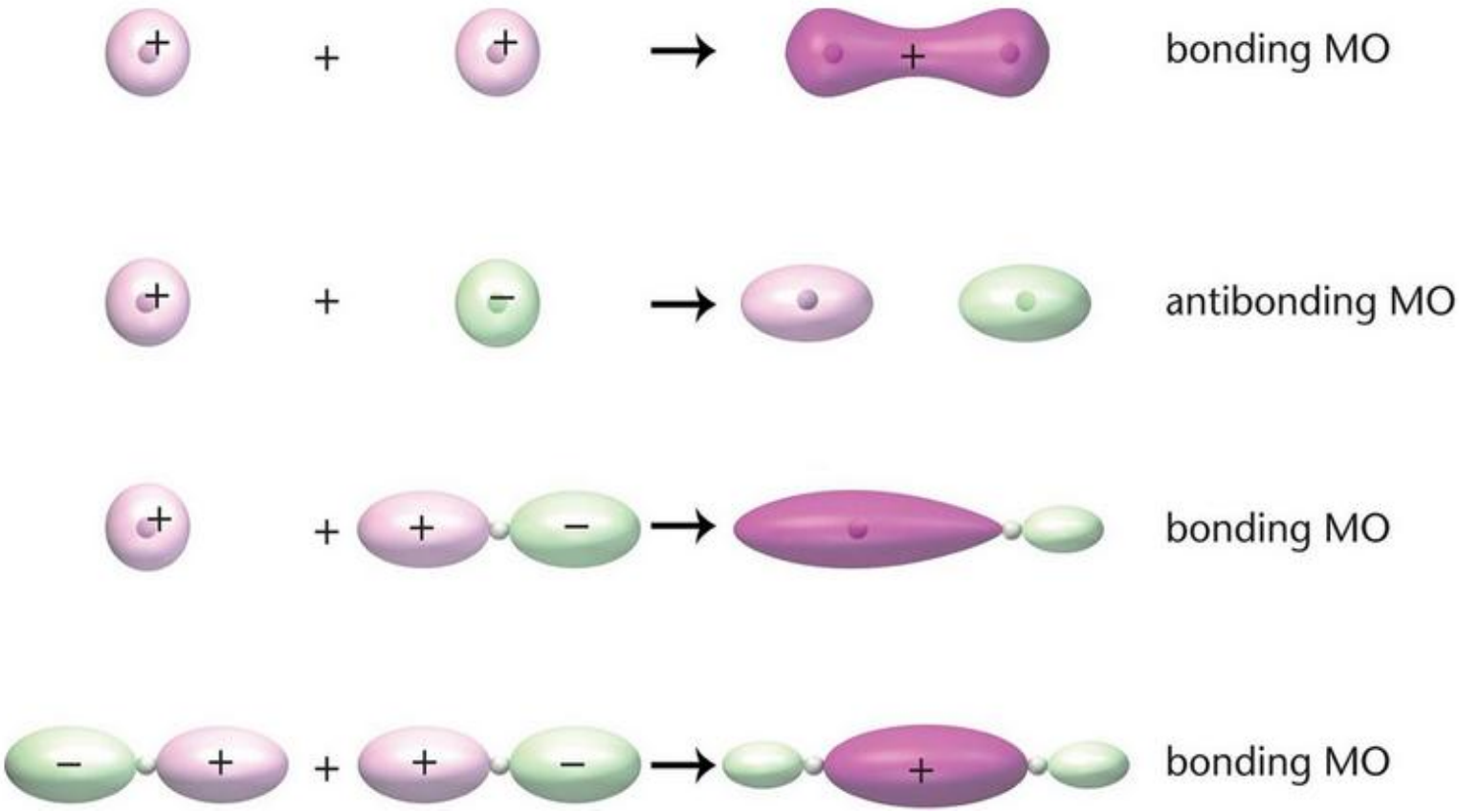


Molecular Orbital (MO) Theory

Molecular orbital (**MO**) **theory:** a method describing the electronic structure of molecules using quantum mechanics.

شرحت مسبقاً في الكيمياء العامة، يرجى المراجعة والاستفسار في حال وجود نقاط غير مفهومة

σ bonds



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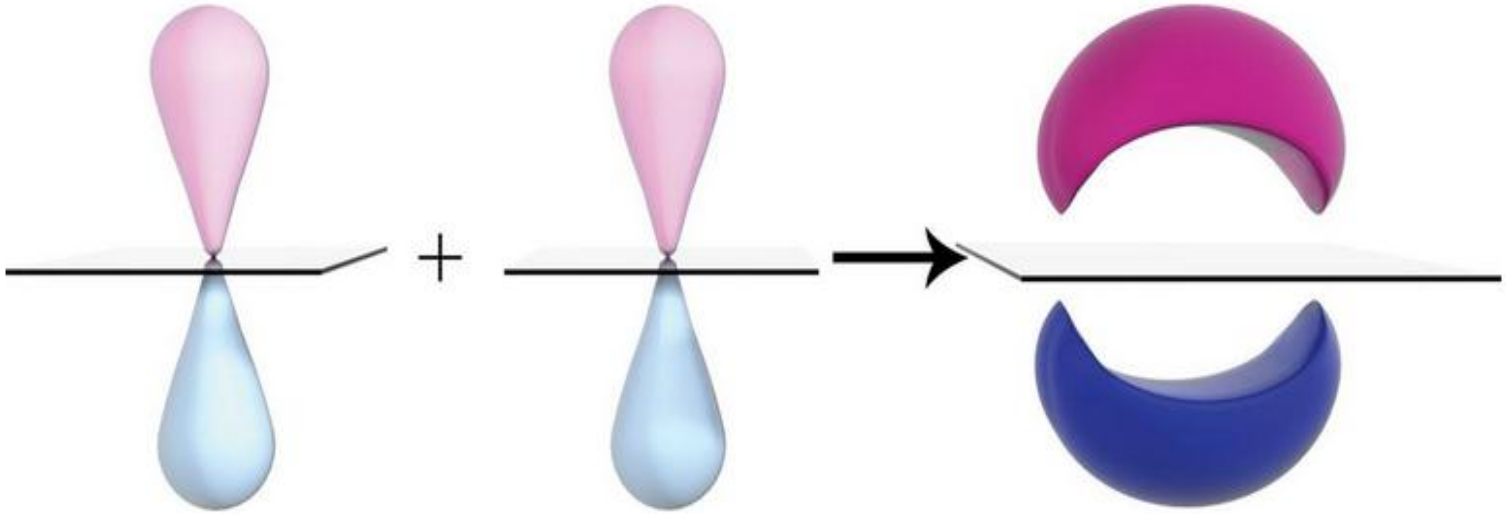
π bonds

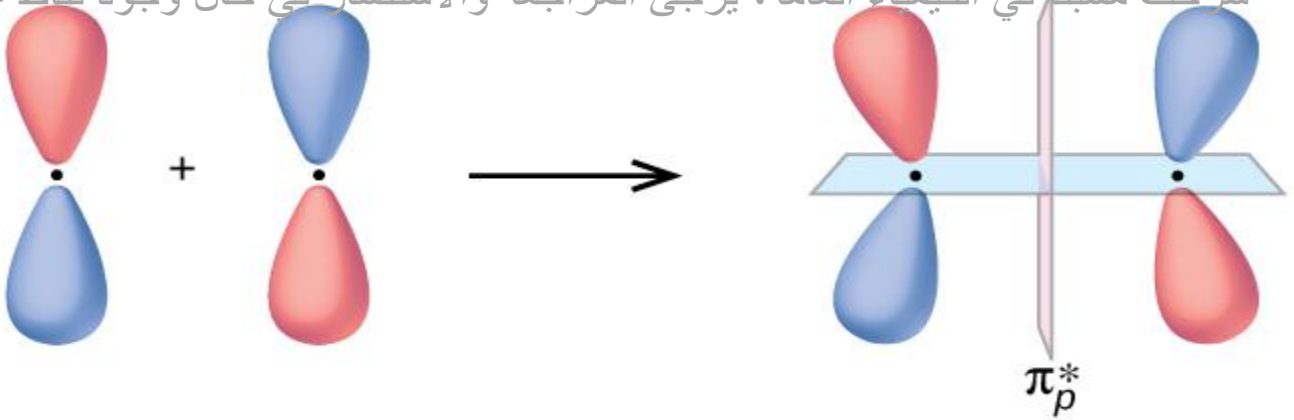


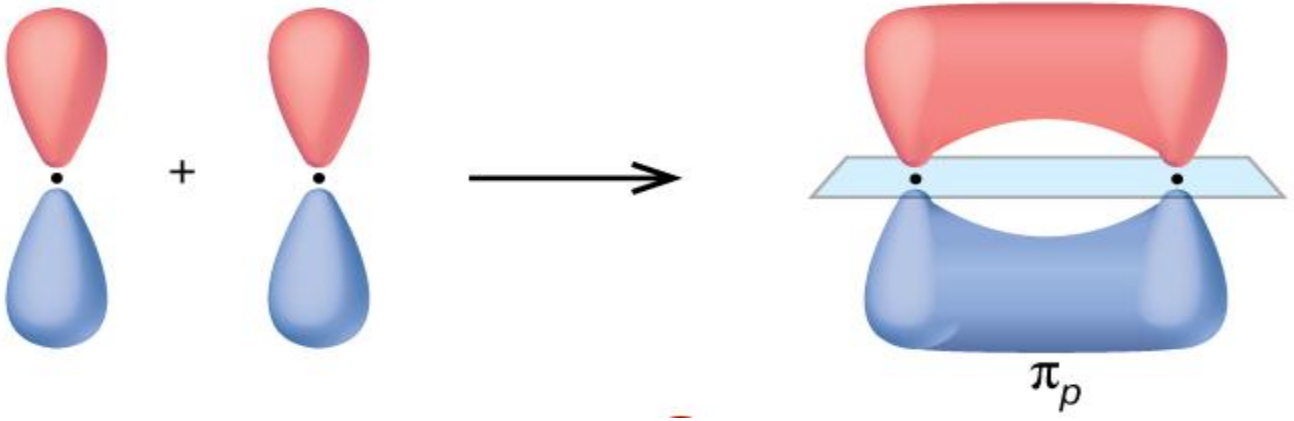
Molecular Orbital (MO) Theory

Types of orbitals

1- Bonding orbitals: signs are the same, a lower-energy (more stable).

2- Antibonding orbitals: signs are different, a higher energy (less stable).





Molecular Orbital (MO) Theory

Molecular orbital (**MO**) **theory:** a method describing the electronic structure of molecules using quantum mechanics.

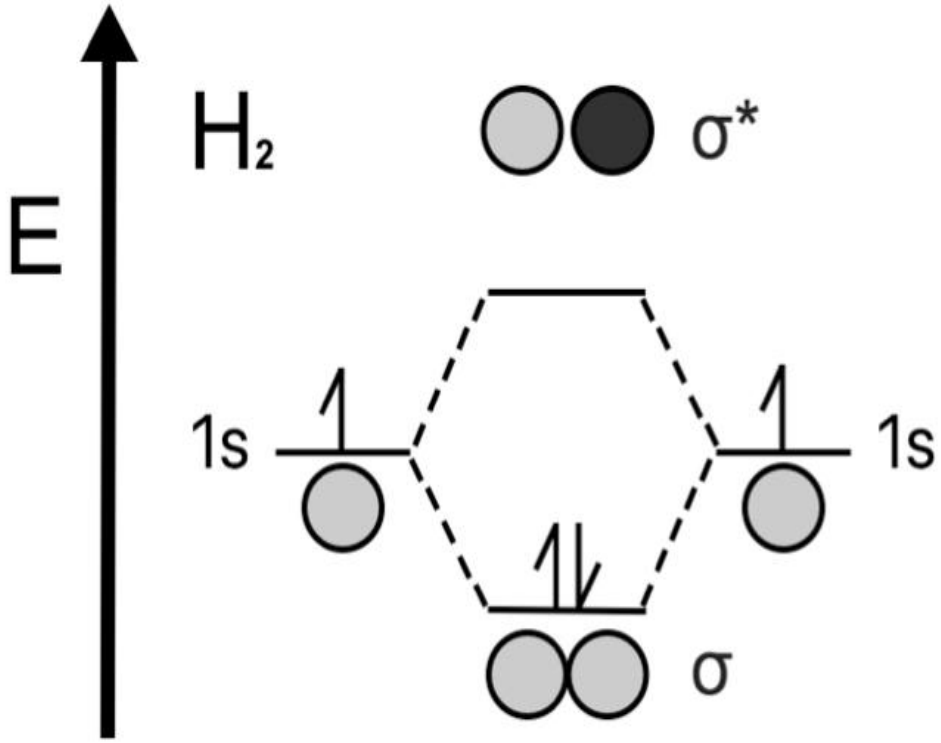
Types of orbitals

1. **Bonding orbitals,** σ π
2. **Antibonding orbitals,** σ^* π^*
3. **Non-bonding orbitals:** are the equivalent in molecular orbital theory of the Lone Pairs in Lewis structures. (n)

شرحت مسبقاً في الكيمياء العامة، يرجى المراجعة والاستفسار في حال وجود نقاط غير مفهومة

Molecular Orbital (MO) Theory

MO diagram

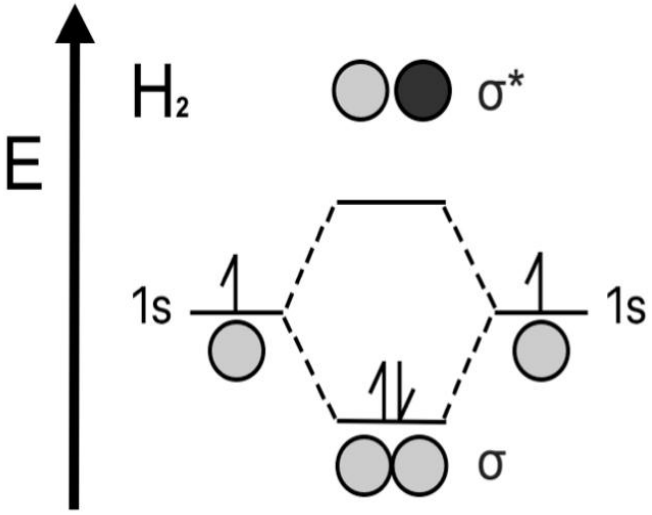


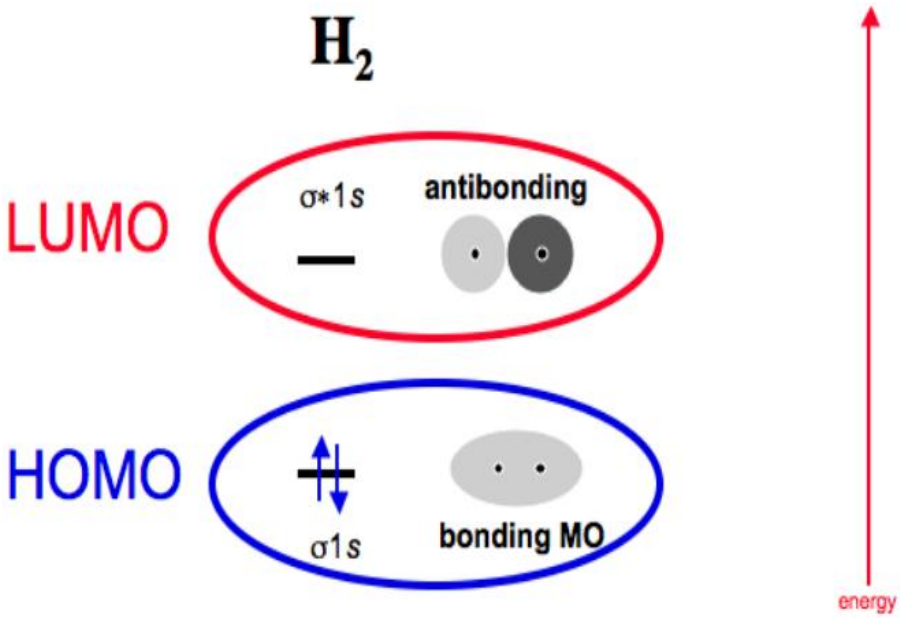
شرحت مسبقاً في الكيمياء العامة، يرجى المراجعة والاستفسار في حال وجود نقاط غير مفهومة

$$\text{bond order} = \frac{(\text{number of bonding electrons}) - (\text{number of antibonding electrons})}{2}$$

Molecular Orbital (MO) Theory

MO diagram





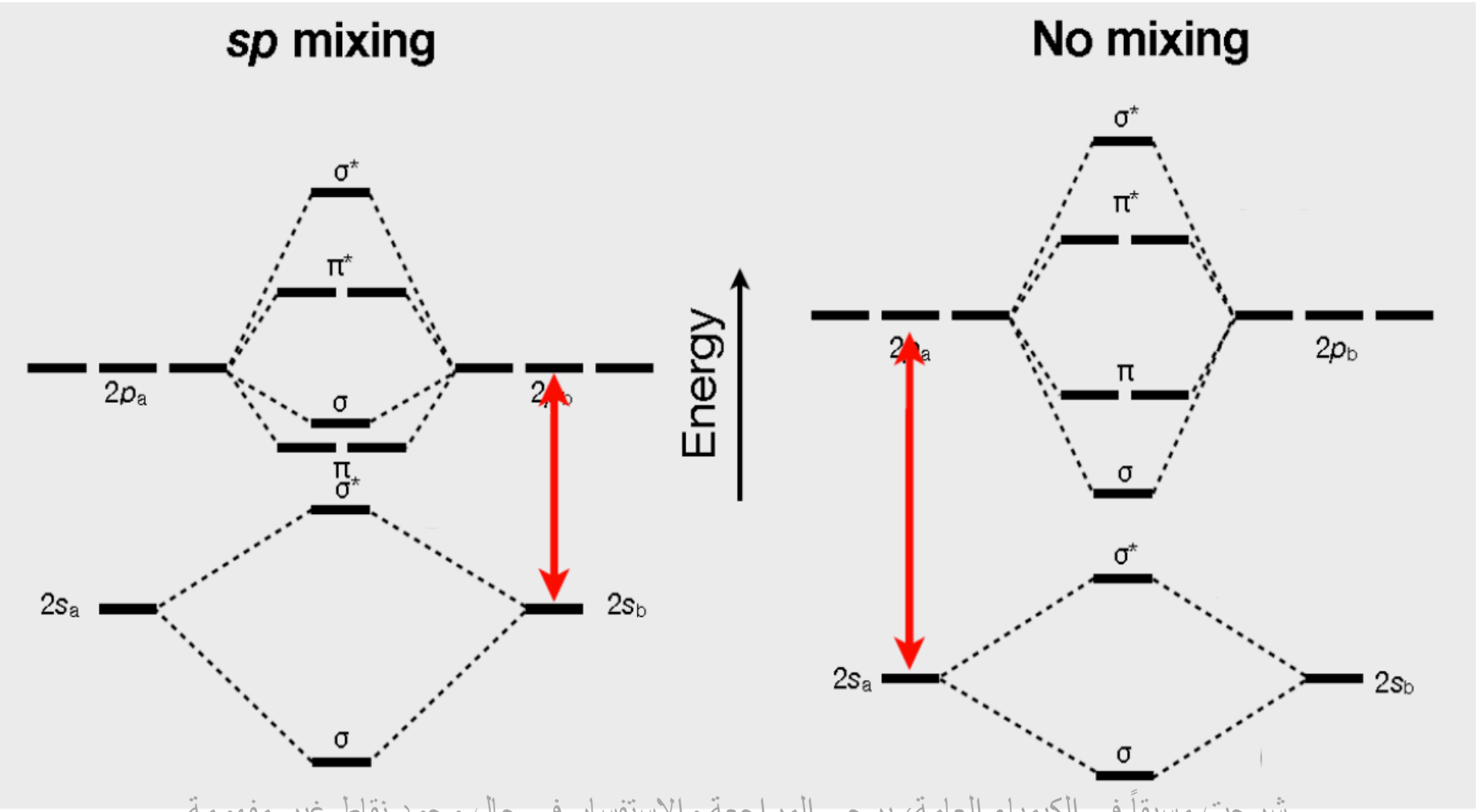
HOMO: the highest occupied molecular orbital,

LUMO: the lowest unoccupied molecular orbital.

شرحت مسبقاً في الكيمياء العامة، يرجى المراجعة والاستفسار في حال وجود نقاط غير مفهومة

SOMO: Semi Occupied Molecular Orbital. (O_2, B_2)

Molecular Orbital (MO) Theory



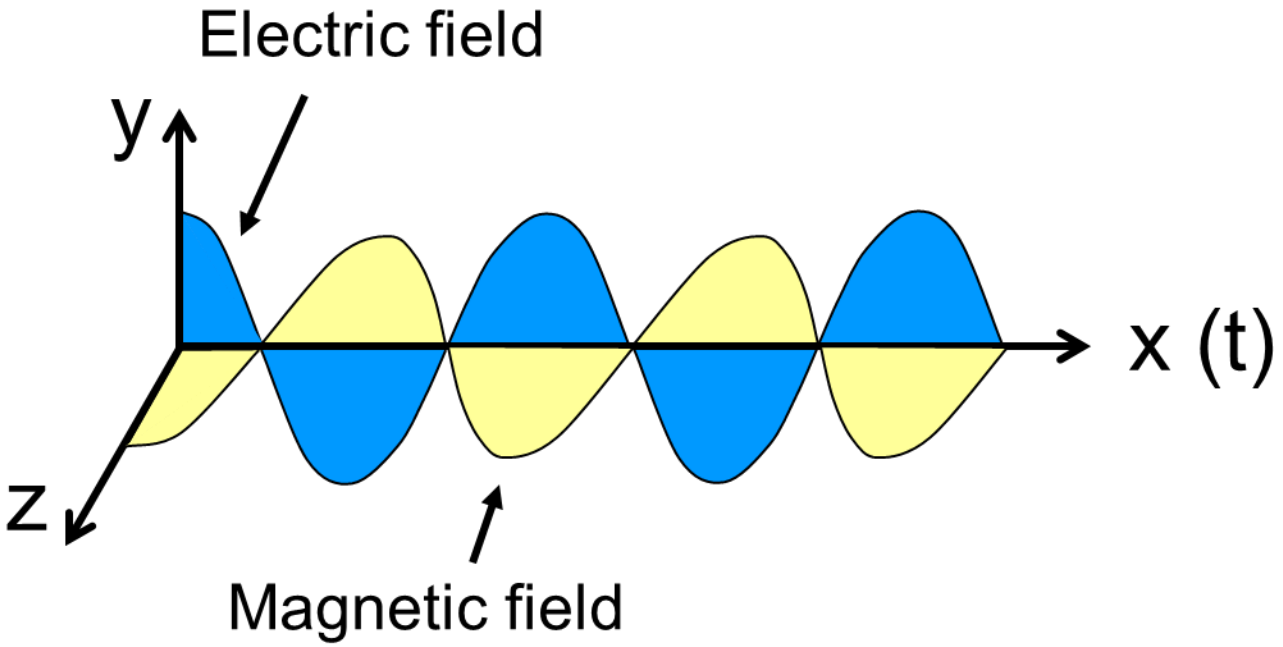
Li_2, Be_2, B_2, C_2, N_2

O_2, F_2, Ne_2

Spectrophotometry

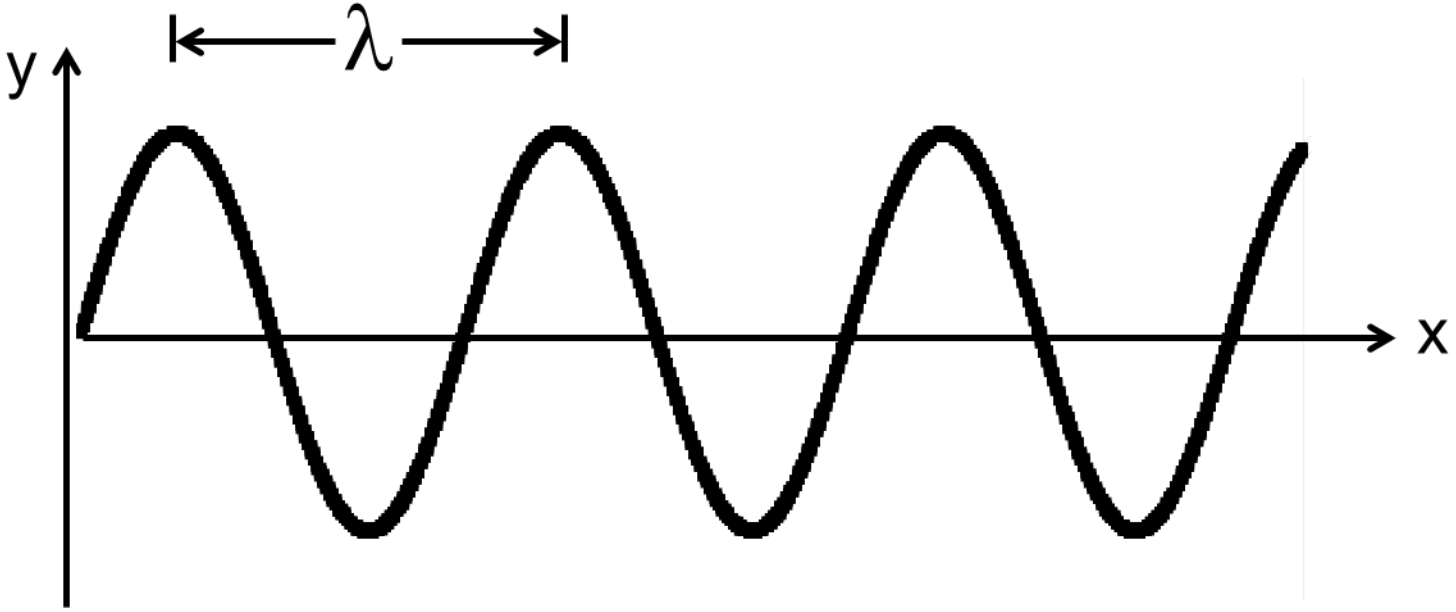
Spectrophotometry: Any procedure that uses light to measure the concentration of a chemical species.

- Light is composed of perpendicular, oscillating electric and magnetic fields



Properties of Light

- **Wavelength (λ):** crest-to-crest (or trough-to-trough) distance between waves, generally measured in nm



- Light described as “waves”

Properties of Light

- **Period (P, T):** the time required for one cycle to pass a fixed point in space, (sec)

$$P = 1/\nu$$

$$\lambda = c\ P$$

- **Frequency (ν):** number of complete oscillations the wave makes each second (1/s, s^{-1}, Hz)

$$\nu = c/\lambda$$

c ≡ speed of light (in vacuum) = 2.998×10^8 m/s

- Units for above equation: $(s^{-1})=(m/s)\ /(m)$

Properties of Light

- **Refractive index (n):** measure of angle at which light is bent
- Speed of light in a medium other than a vacuum= c/n

(n = 1 in a vacuum)

n=1.33 Water

n=1.36 Ethanol

n=2.15 ZrO_2

n=2.42 Diamond

Properties of Light

- **Photons ($h\nu$):** energetic particles of light

$$E = h\nu$$

$$E = hc/\lambda = hc\tilde{\nu}$$

$h \equiv$ Planck's constant = 6.626×10^{-34} J•s

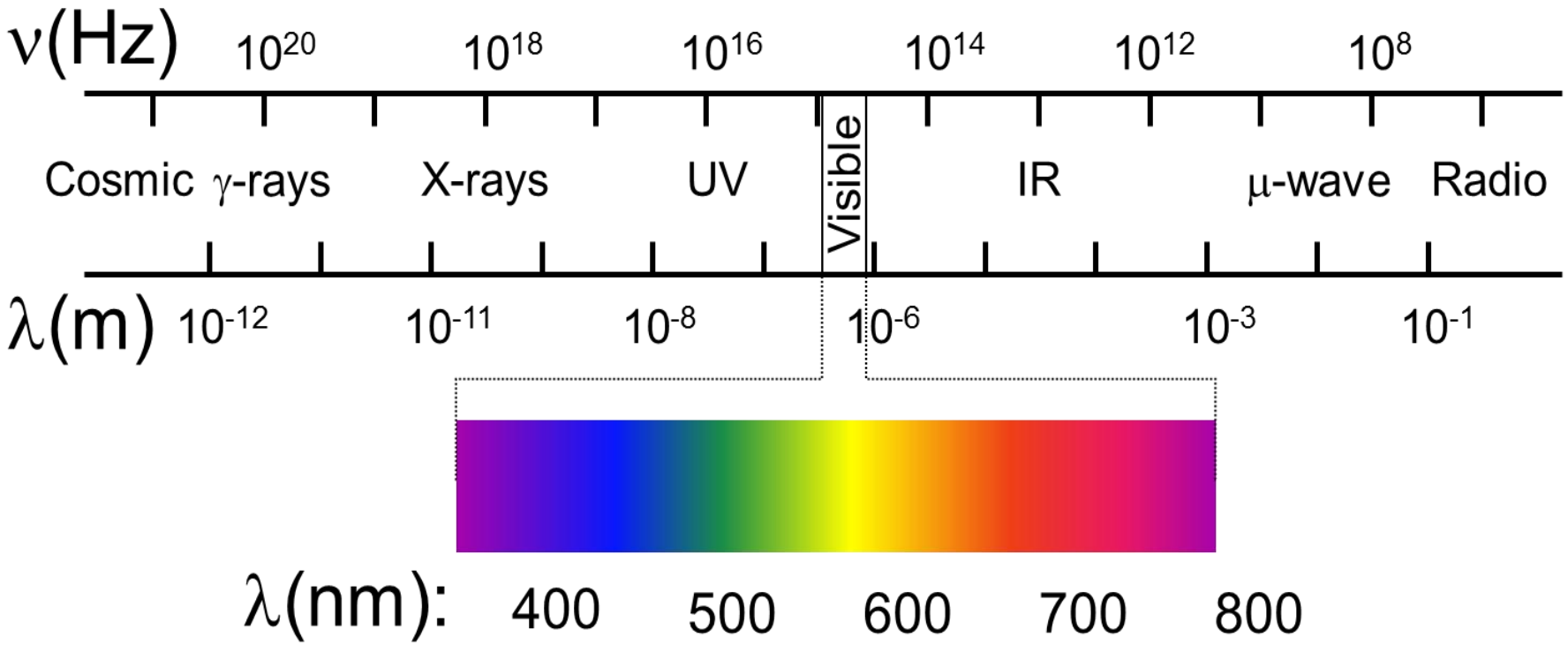
$\tilde{\nu} \equiv$ wavenumber ($1/\lambda$)

Electromagnetic Spectrum

- Compare energy of red and green light:

$$E = hc/\lambda$$

$$\lambda_{red} > \lambda_{blue} \quad \text{so} \quad E_{red} < E_{blue}$$

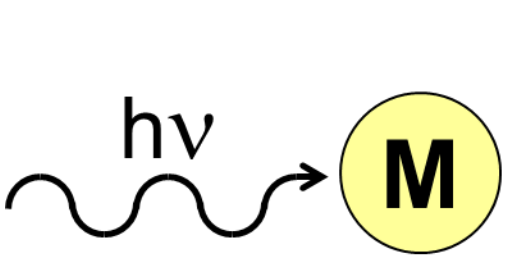


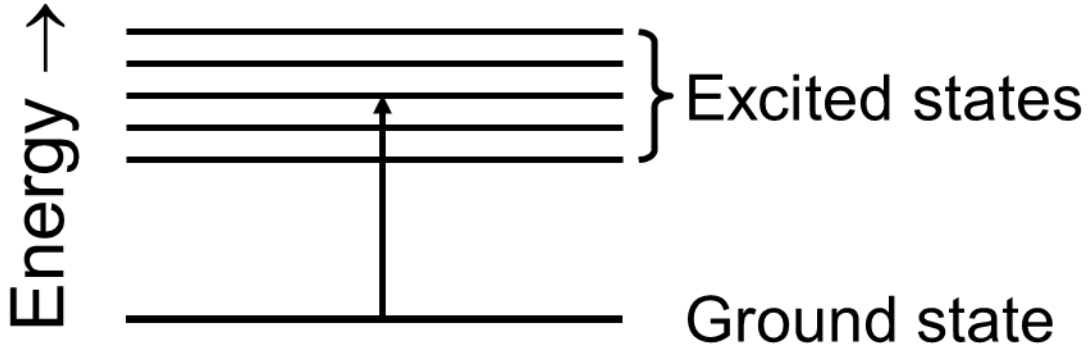
Absorption of light

- Molecules absorb photons with energy (E)

$$E = h\nu$$

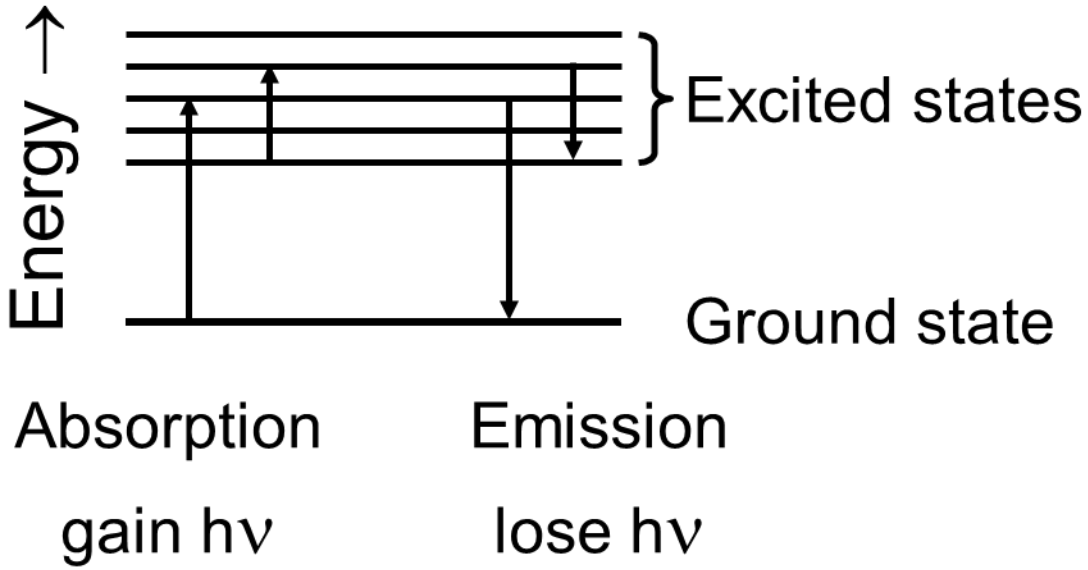
- Molecules gain that energy (E) when they absorb photons
- M is promoted from the ground state to an excited state.





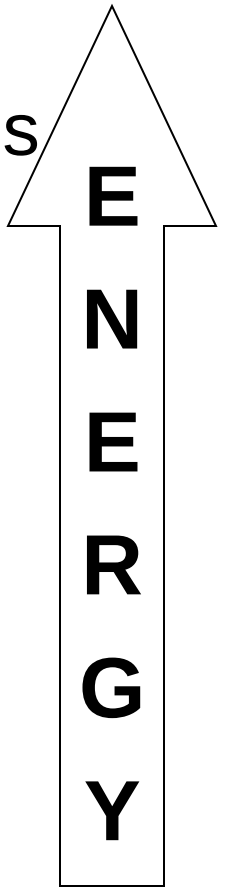
Absorption of light

- Molecules gain energy when they absorb photons
- Molecules lose energy when they emit photons



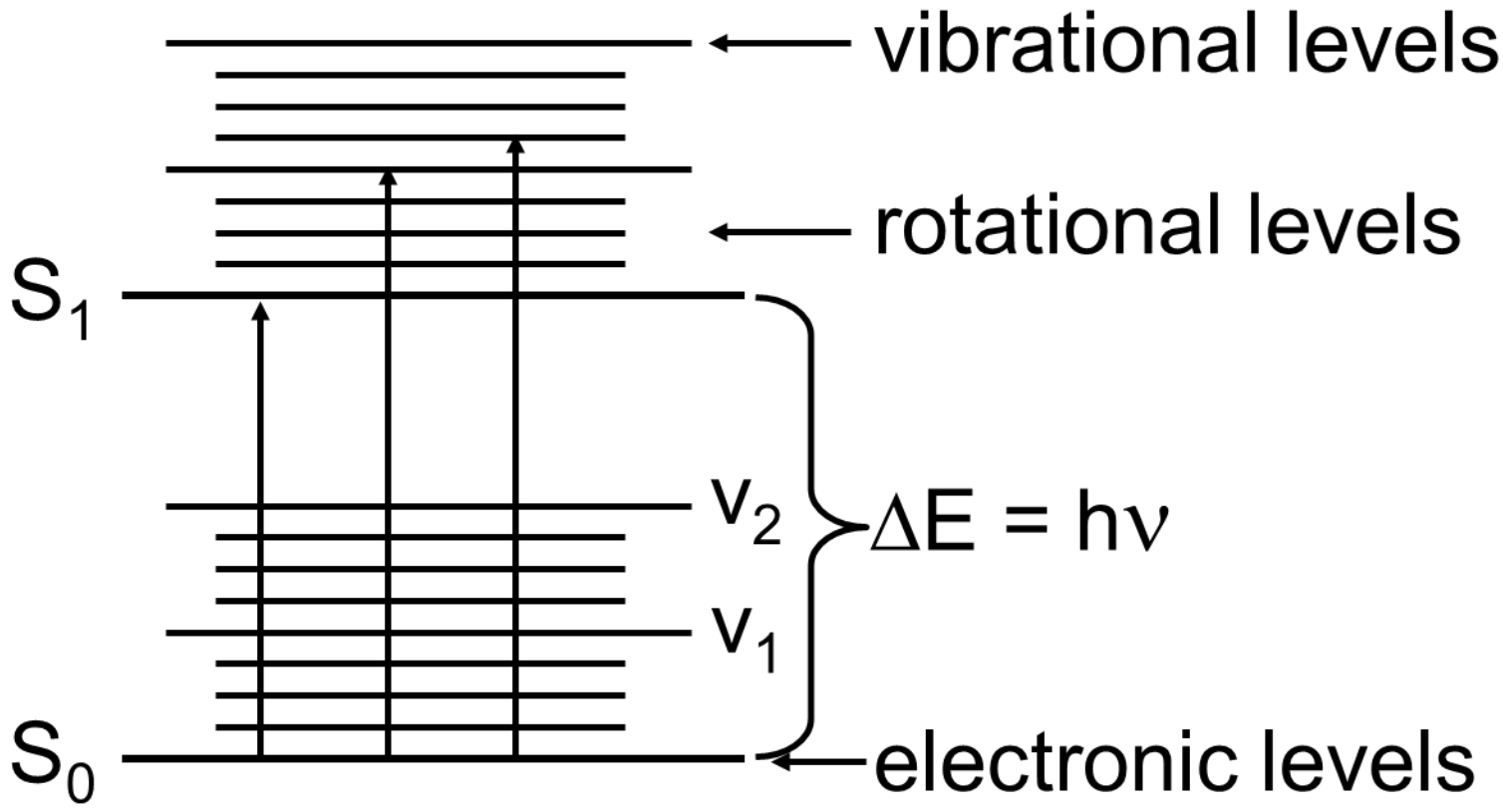
Types of electromagnetic radiation (light)

- X-ray light
  - promotes core $(e^-)_s$ to higher energy orbitals
  - breaks chemical bonds and ionizes molecules
- Ultraviolet and visible light (UV-VIS)
  - promotes valence $(e^-)_s$ to higher energy orbitals
- Infrared light (IR)
  - stimulates vibrations of molecules
- Microwaves
  - stimulates rotational motion of molecules



Energy levels

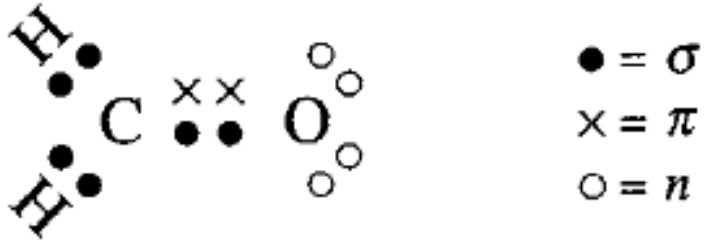
- **Quantized:** discrete levels, not continuous
- Energy states are “quantized”

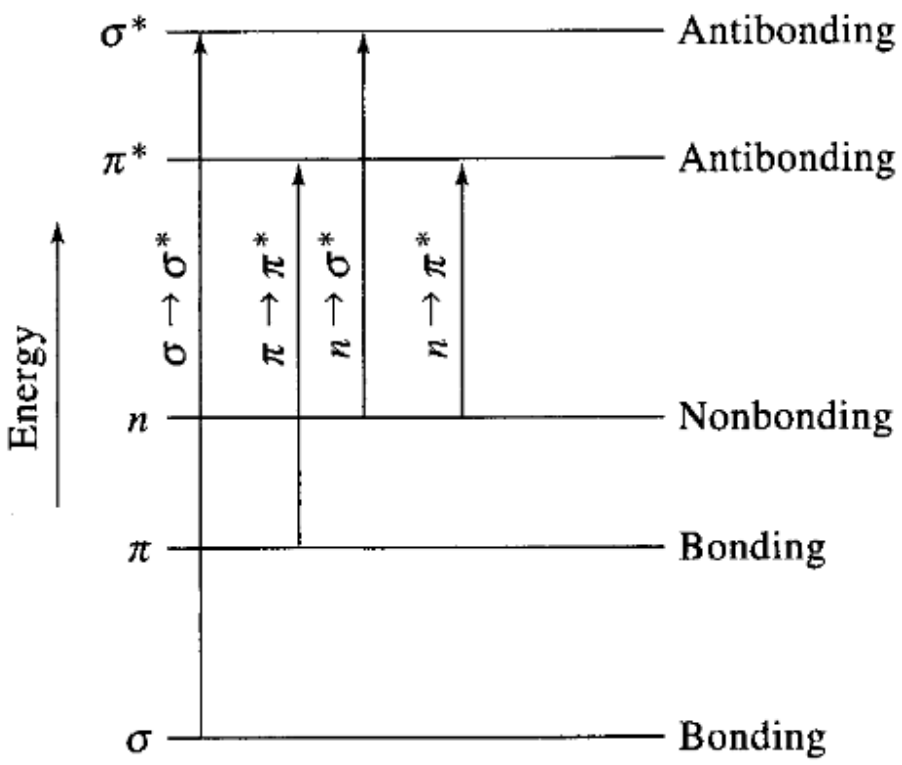


Energy levels

σ, π, and lone pair (n) electrons.

The σ, π, and lone pair (n) electrons can all undergo excitation from ground state to excited energy states.





The four transitions are:

σ to σ*, π to π*, n to σ*, and n to π*.

The promoted electrons are usually bonding electrons.

What happens when a molecule absorbs light?

Three basic processes by which a molecule can absorb radiation:

- Rotational transition: the molecule rotates about various axes, the energy of rotation being at definite energy levels.
- Vibrational transition: the atoms or groups of atoms within a molecule vibrate relative to each other, and the energy of this vibration occurs at definite quantized levels.
- Electronic transition: the electrons of a molecule may be raised to a higher level of electron energy, corresponding to an electronic transition.

What happens when a molecule absorbs light?

The relative energy levels of the three transition processes are in the order:

Electronic > Vibrational > Rotational

Each are about an order of magnitude different in its energy level.

- Rotational transitions: very low energies (long wavelengths: Microwave or far-infrared region).
- Vibrational transitions: require higher energies in the near-infrared region.
- Electronic transitions: require still higher energies (in the visible and ultraviolet regions).

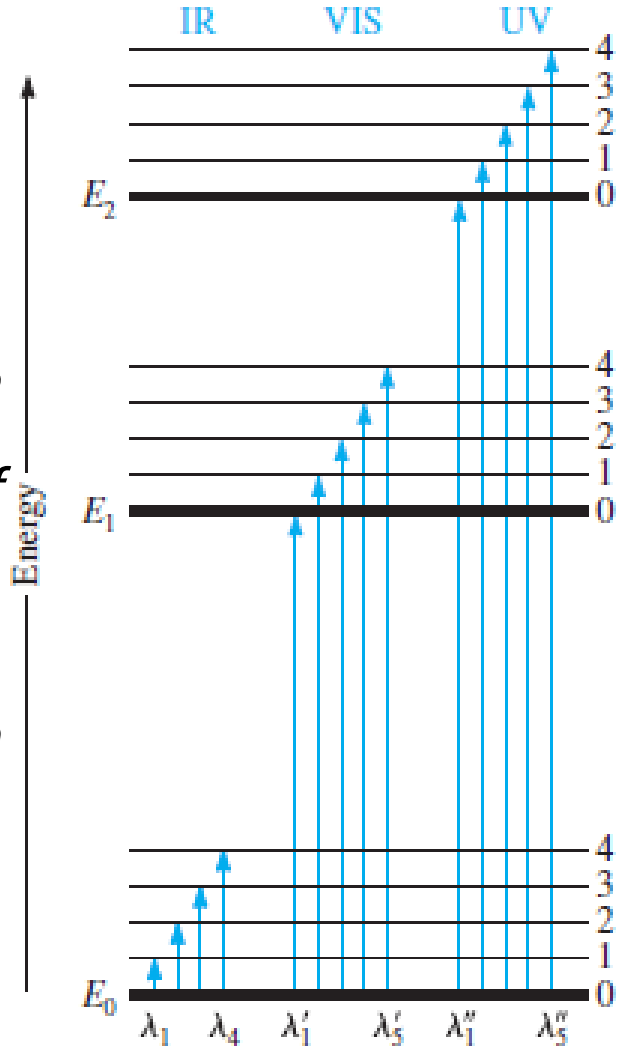
What happens when a molecule absorbs light?

Energy level diagram

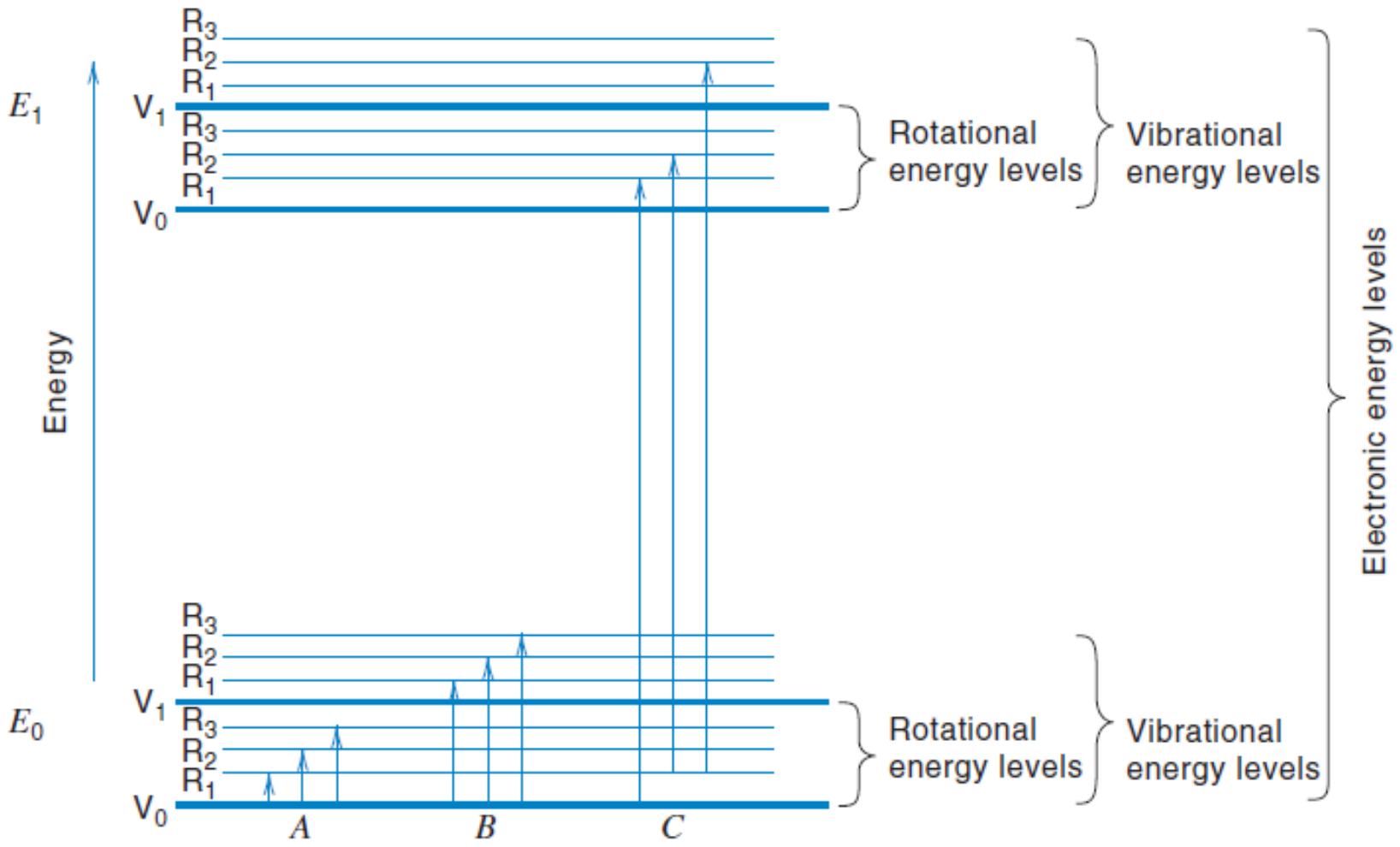
Energy changes occurring during absorption of (IR), (VIS), and (UV) radiation by a molecular species.

Some molecules : transition from E_0 *to* E_1 *may require UV radiation instead of* visible radiation.

Other molecules: the transition from E_0 *to* E_2 *may occur* with visible radiation instead of UV radiation.



What happens when a molecule absorbs light?



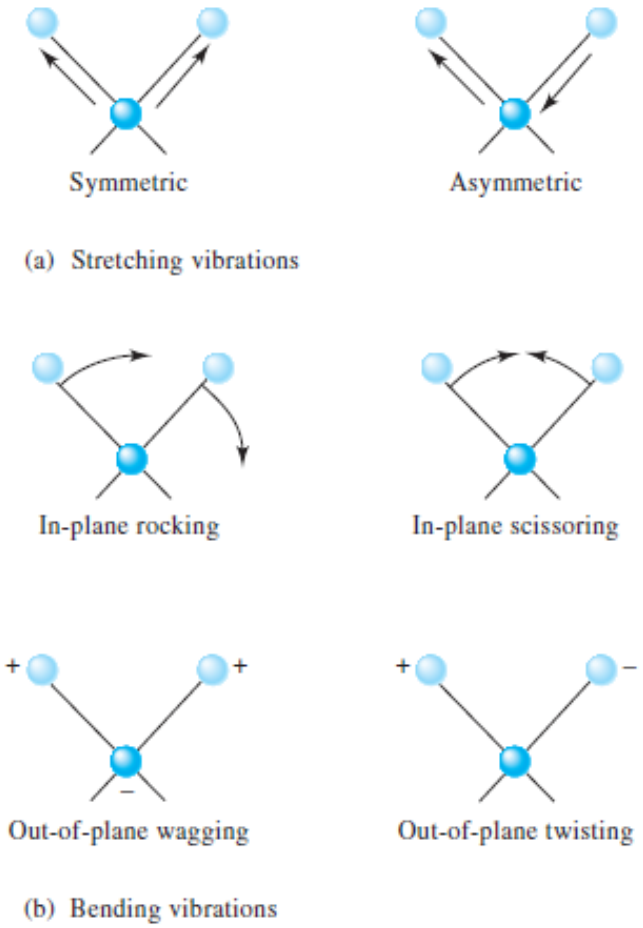
E_0 is electronic ground state **E_1** is first electronic excited state

What happens when a molecule absorbs light?

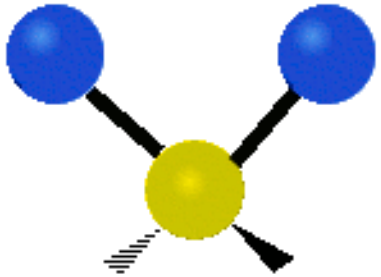
Infrared Absorption:

IR generally is not energetic enough to cause electronic transitions, but it can induce transitions in the vibrational and rotational states associated with the ground electronic state of the molecule.

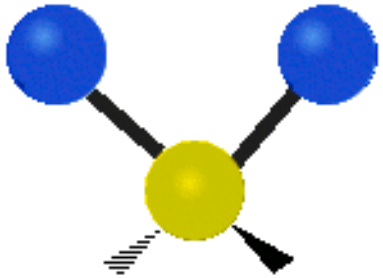
For absorption to occur, the radiation source has to emit frequencies corresponding exactly to the energies indicated by the lengths of the four arrows.



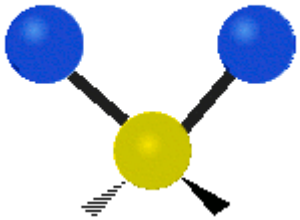
Molecular vibration



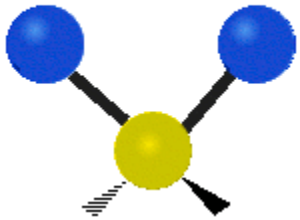
Symmetrical stretching



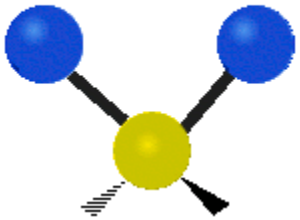
Asymmetrical stretching



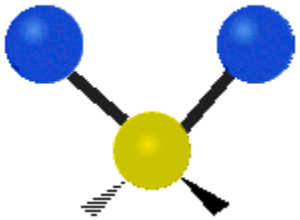
Twisting



Wagging



Rocking



Scissoring

Exercice

- By how many kJ per mole is the energy of O_2 increased when it absorbs UV radiation with a λ of 147 nm?
- New energy: E_2
- Original energy: E_1

$E_2 - E_1$ = energy of photon absorbed

$$\Delta E = h\nu = \frac{hc}{\lambda}$$

Example Problem

$$\Delta E = h\nu = \frac{hc}{\lambda}$$

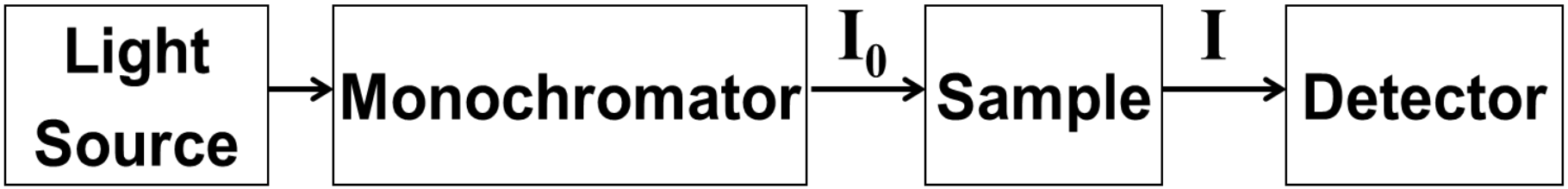
$$\Delta E = \frac{(6.626\times10^{-34}\,\mathrm{J\cdot s})(2.998\times10^{8}\,\mathrm{m/s})}{147\mathrm{nm}}\times\frac{10^{9}\mathrm{nm}}{\mathrm{m}}$$

$$= 1.35\times10^{-18}\,\mathrm{J/molecule}$$

$$\frac{1.35\times10^{-18}\,\mathrm{J}}{\mathrm{molecule}}\times\frac{6.022\times10^{23}\,\mathrm{molecules}}{\mathrm{mol}}\times\frac{1\mathrm{kJ}}{1000\mathrm{J}}$$

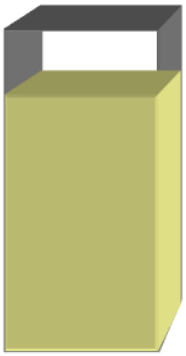
$$\boxed{= 814\mathrm{kJ/mol}}$$

Measuring Absorption of Light

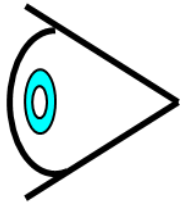


source may contain many λs

selects λ that analyte will absorb



sample absorbs radiation



Detector looks for amount of Radiation **not** absorbed

Measuring Absorption of Light

- Detector measures (I)
- Amount of light transmitted through sample is what is measured
- **Transmittance (T):** fraction of original light that passes through sample
  - Absorbance is measured **INDIRECTLY**

$$T = \frac{I}{I_0} = \frac{\text{radiant power not absorbed by sample}}{\text{incident radiant power}}$$

$$\%T = 100T$$

Measuring Absorption of Light

$$0 < T < 1$$

$$0 < \%T < 100$$

- Absorbance (A) is related to T:

$$A = -\log T = \log\left(\frac{1}{T}\right) = \log\left(\frac{I_0}{I}\right)$$

$$A = \log\left(\frac{100}{\%T}\right) = \log 100 - \log \%T$$

$$\boxed{A = 2 - \log \%T}$$

Measuring Absorption of Light

$$A = -\log T$$

%T=70 %

T=0.7

A=-log T

A=-log 0.7

A=0.155

Absorbance vs. Transmittance

- When (I) decreases, (A) increases
  - less radiant power (light) is reaching the detector because the sample is absorbing light
- T and A are dimensionless (although sometimes “absorbance units (Au)” are mentioned)

I/I_0	%T	A
1	100	0
0.1	10	1
0.01	1	2

Absorbance vs. Reflection

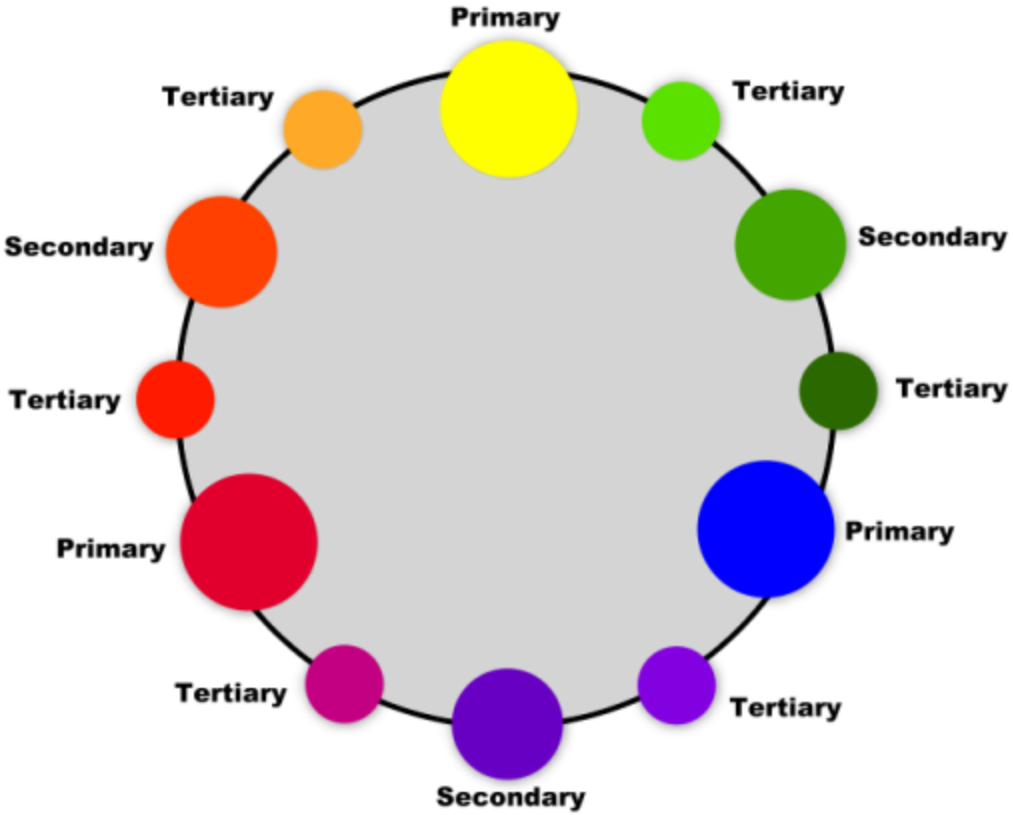
- When a molecule absorbs different λs of white (visible) light, our eyes see the reflected λs (the non-absorbed λs)

Table 19-1

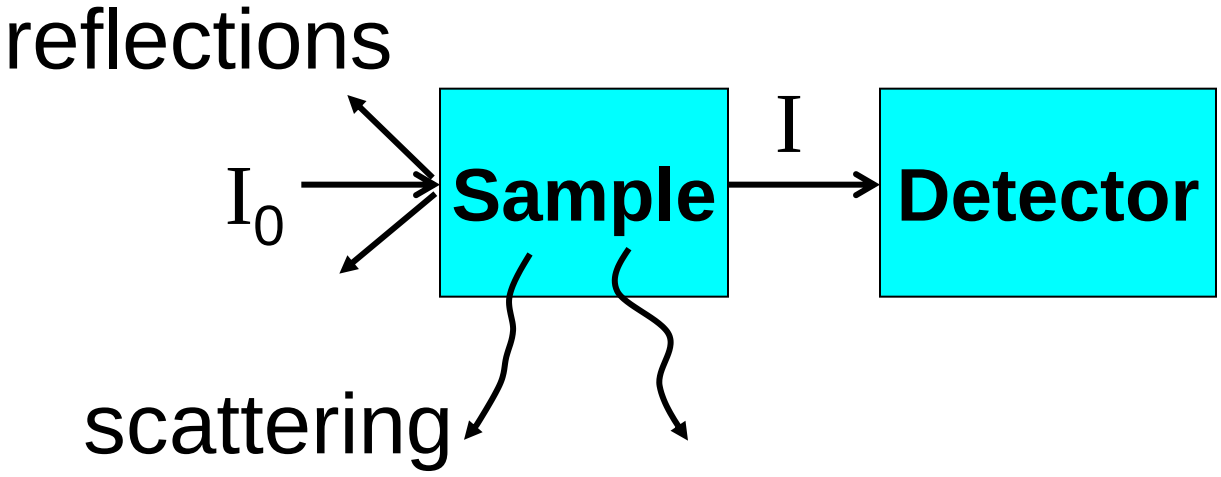
λ of Max. Absorption	Color Absorbed	Color Observed
380-420	violet	green-yellow
420-440	violet-blue	yellow
440-470	blue	orange
470-500	blue-green	red
500-520	green	purple
520-550	yellow-green	violet
550-580	yellow	violet-blue
580-620	orange	blue
620-680	red	blue-green
680-780	purple	green

Blue Blocker Sunglasses

- The infomercial claims that “harmful blue light” is blocked from damaging your eyes.
- Why are they orange?



Measuring Absorption of Light



$$I_{(\text{for absorption by sample})} = I_0 - I_{(\text{reflected})} - I_{(\text{scattered})}$$

- **Reference blank:** solution containing all components of a sample except analyte

$$A_{(\text{analyte})} = A_{(\text{measured})} - A_{(\text{reference blank})}$$

Absorbance

- **Spectrophotometry:** Any procedure that uses light to measure the concentration of a chemical species
- **Beer's Law: (Beer–Lambert–Bouguer law)**

$$A = \varepsilon bc$$

$\varepsilon \equiv$ Molar absorptivity (or extinction coefficient)

$b \equiv$ path length light travels through cuvet

$c \equiv$ concentration of analyte

- Absorbance is directly proportional to concentration

Beer's Law

- Best applied when $c \leq 0.01$ M
  - when $c > 0.01$ M, solute molecules influence one another because they are closer together
  - physical properties of molecules change when they are close together
  - physical property that will change relevant to our discussion of Beer's Law: ε

Beer's Law

Molar absorptivity (ε): characteristic of a substance that tells how much light is absorbed at a particular wavelength (λ)

- ε is λ-dependent AND analyte-dependent because different analytes absorb different amounts of light at different λs

Pathlength (b): width of cuvet; dependent on instrumental setup

$$A = \varepsilon bc = (M^{-1}\ cm^{-1})(cm)(M)$$

<u>A is dimensionless</u>

Example Problem-1

- A 3.15 x 10^{-6} M solution of a colored complex exhibited an absorbance of 0.267 at 635 nm in a 1.000 cm cuvet. A blank solution had an absorbance of 0.019. Find the molar absorptivity of the colored complex.

$$A = \varepsilon bc$$

$$\varepsilon = \frac{A}{bc} = \frac{(0.267 - 0.019)}{(1.000\text{cm})(3.15 \times 10^{-6}\text{M})}$$

$$\varepsilon = 7.87 \times 10^{4}\text{M}^{-1}\text{cm}^{-1}$$

Example Problem-2

A 7.25 x 10^{-5} M solution of potassium permanganate has a transmittance of 44.1% when measured in a 2.10 cm cell at a wavelength of 525 nm. Calculate (a) the absorbance of this solution and (b) the molar absorptivity of $KMnO_4$.

Solution

(a) $$A = -\log T = -\log(0.441)$$

$$A = -(-0.356) = 0.356$$

(b) $$\varepsilon = \frac{A}{bc} = \frac{(0.356)}{(2.1cm)(7.25\times10^{-5}M)}$$

$$\varepsilon = 2.34\times10^{3} Lmol^{-1}cm^{-1}$$

Example Problem-3

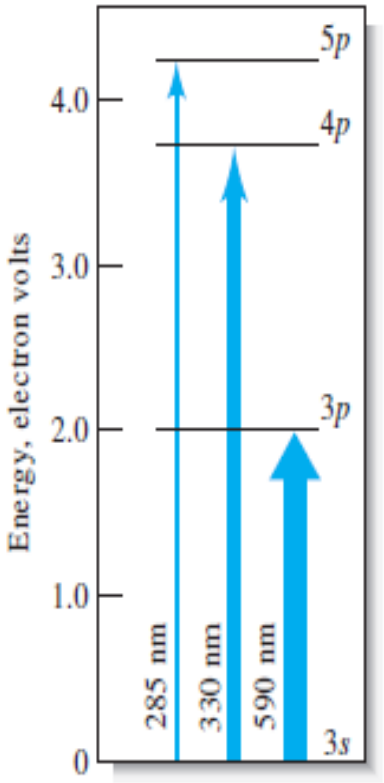
The energy difference between the 3*p* and the 3*s* orbitals in is 2.107 eV. Calculate the wavelength of radiation that would be absorbed in exciting the 3*s* electron to the 3*p* state (1 eV = 1.60 * 10-19 J).

Solution

$$E = \frac{hc}{\lambda} \Rightarrow \lambda = \frac{hc}{E}$$

$$\lambda = \frac{6.63\times10^{-34}\,Js \times 3.00\times10^{10}\,cm/s \times 10^{7}\,nm/cm}{2.107eV \times 1.60\times10^{-19}\,J/eV}$$

$$\lambda = 590nm$$



Example Problem-4

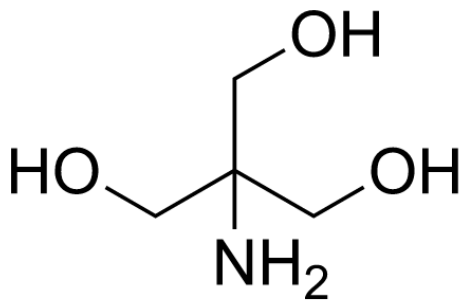
An unknown amine (0.1155 g) is dissolved in water and diluted to 100 mL. A 1-mL aliquot of this is diluted to 250mL for measurement. If this final solution exhibits an absorbance of 0.454 at 359 nm using a 1.00-cm cell, what is the Molecular weight of the amine. $\varepsilon = 1.25 \times 10^4$

أذيب (0.1155 غ) من أمين مجهول التركيب في 100 مل من الماء المقطر. مُدد 1 مل من المحلول إلى 250 مل من أجل القياس باستعمال المطيافية الضوئية.
إذا كانت امتصاصية المحلول الممدد (0.454) عند طول موجة 359 نانومتر، باستعمال خلية (1.00 سم)، أحسب الوزن الجزيئي للأمين.

Example Problem-4

$$A = \varepsilon bc$$

$$c_1 = \frac{A}{b\varepsilon} = \frac{(0.454)}{(1.00)(1.25\times10^{4})} = 3.63\times10^{-5}\,M$$



Trisamine

$$C_1.V_1 = C.V \qquad 3.63\times10^{-5}\times100 = C\times1$$

$$C = 3.63\times10^{-5}\times100 = 3.63\times10^{-3}\,M$$

$$M = \frac{n}{V_{(L)}} \qquad n = M.V$$

$$n = M.V = 3.63\times10^{-3}\times0.250 = 9.08\times10^{-4}\,mol$$

$$n = \frac{m}{M_w} \qquad M_w = \frac{m}{n} = \frac{0.1155}{0.908\times10^{-4}} = 127.2\,g/mol$$

Using a spectrophotometer

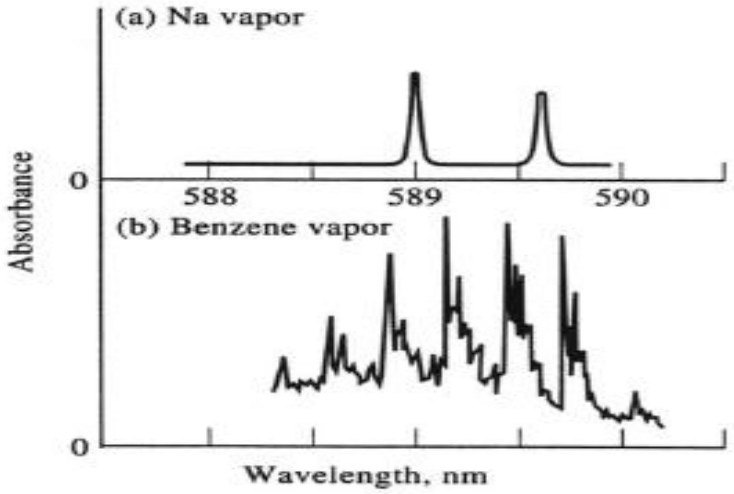
- Picking the λ (the source of the light)
  - Choose the λ at which the analyte absorbs the most (λ_{max})
    - measurement is most sensitive at λ_{max}
- Keep samples clean and dust free
- Analyte solution should absorb in the range of $0.4 < A < 0.9$
  - dilute solution if it is too concentrated
    - reduce c to reduce A
  - use cuvet with longer pathlength
    - increase b to increase A

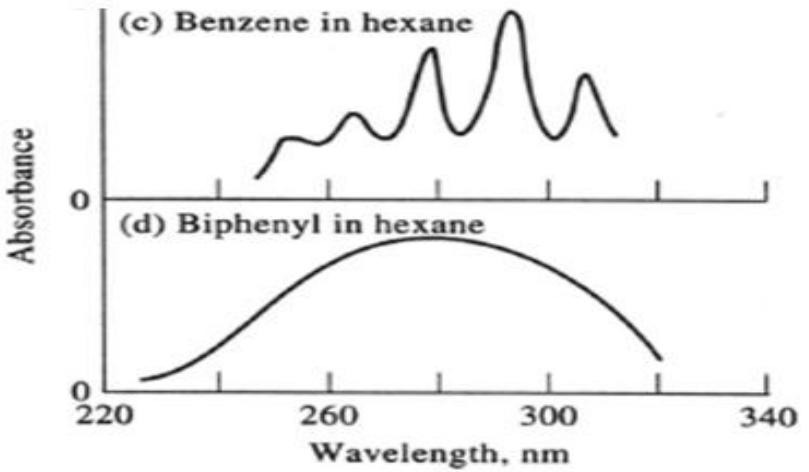
Why should 0.4 < A < 0.9?

- Consider when A < 0.4
  - I is almost as large as I_0
  - difficult to see a small difference between two large numbers
- Consider when A > 0.9
  - I is very very small
  - difficult to see a small amount of light
  - stray light reaching the detector could compete with I
- Adjust experimental parameters to keep A in an intermediate region

Spectra models

Absorber	Phase	Notes/transition types
(a) Atom	**Gas**	Extremely narrow lines / electronic
(b) Molecule	**Gas**	Fine structure due to electronic + rotational + Vibrational
(c) Molecule/small	**Solution**	Broad bands + some fine structure / electronic + vibrational
(d) Molecule/Larger	**Solution**	Broad bands electronic only resolved





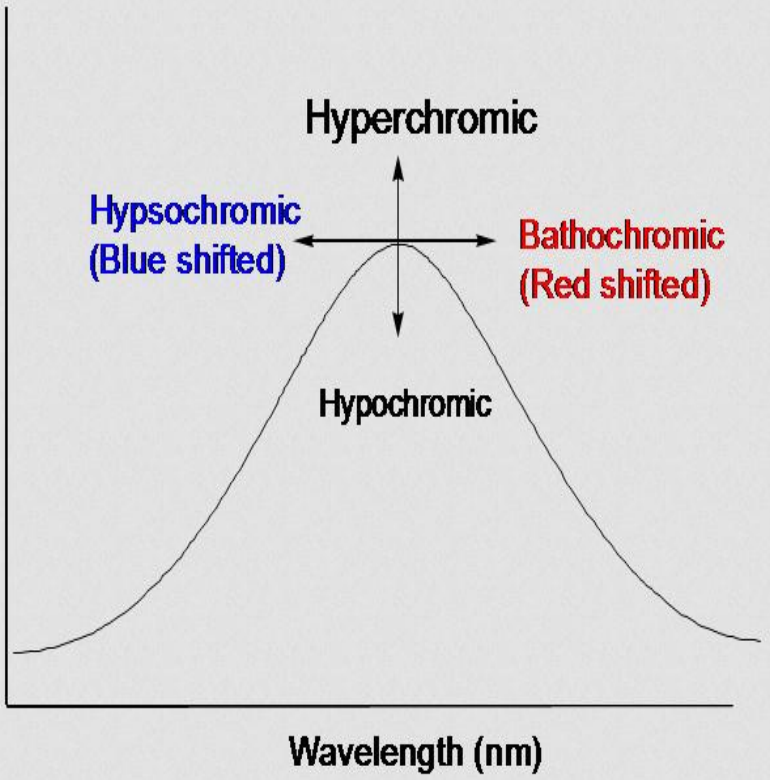
Terminology for absorption shifts

Bathochromic: a shift of a band to lower energy or longer wavelength (Red shift).

Hypsochromic: a shift of a band to higher energy or shorter wavelength (Blue shift).

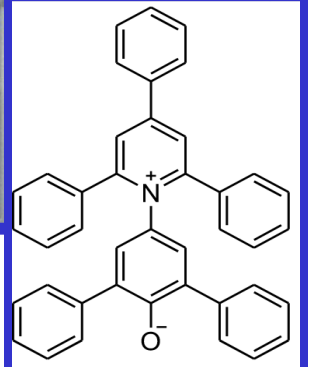
Hyperchromic: an increase in the molar absorptivity.

Hypochromic: an decrease in the molar absorptivity.



The solvatochromic effect is the way the spectrum of a substance varies when the substance is dissolved in a variety of solvents.

Betaine 30: Reichardt's dye



UV/Vis Analysis: Selected Example

Analyte	Method	λ (nm)
total serum protein	reaction with protein, NaOH, and Cu^{2+} produces blue-violet complex	540
serum cholesterol	reaction with Fe^{3+} in presence of isopropanol, acetic acid, and H_2SO_4 produces blue-violet complex	540
uric acid	reaction with phosphotungstic acid produces tungsten blue	710
serum barbiturates	barbiturates are extracted into $CHCl_3$, and then into 0.45 M NaOH	260
glucose	reaction with *o*-toludine at 100 °C produces blue-green complex	630
protein-bound iodine	decompose protein to release iodide; I^- determined by its catalytic effect on redox reaction between Ce^{4+} and As^{3+}	420

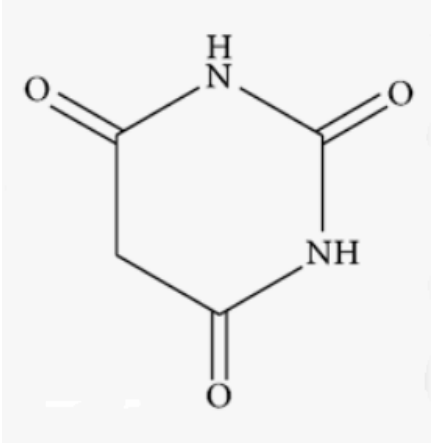
Barbiturates

- ✓ A central nervous system depressant (mild sedation-death).
- ✓ Anxiolytics, hypnotics, and anticonvulsants,
- ✓ physical and psychological addiction,
- ✓ Largely replaced by benzodiazepines,

in the treatment of anxiety and insomnia,

- ✓ Still in use for various purposes:

general anesthesia, epilepsy,
acute migraines, acute tension headaches,
euthanasia, capital punishment,
assisted suicide



Barbituric acid

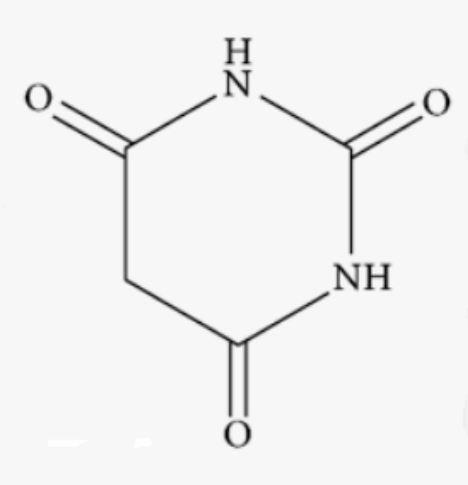
pKa=4.01

Case study-1: determination of serum barbiturates

The analysis of clinical samples is often complicated by the complexity of the sample matrix, which may contribute a significant background absorption at the desired wavelength.

- The determination of serum barbiturates provides one example of how this problem is overcome.

•The barbiturates are extracted from a sample of serum with $CHCl_3$, and extracted from the $CHCl_3$ into 0.45 M NaOH (pH=13).



Barbituric acid
pKa=4.01

Case study-1: determination of serum barbiturates

The absorbance of the aqueous extract is measured at 260 nm and includes contributions from the barbiturates as well as other components extracted from the serum sample.

The pH of the sample is then lowered to approximately 10 by adding NH_4Cl, and the absorbance remeasured. The barbiturates do not absorb at this pH, the absorbance at pH 10 is used to correct the absorbance at pH 13.

$$A_{\text{barb}} = A_{\text{pH 13}} - \left(\frac{V_{\text{samp}} + V_{\text{NH}_4\text{Cl}}}{V_{\text{samp}}} \right) A_{\text{pH 10}}$$

Case study-2: Pharmaceutical Analysis

Many pharmaceutical compounds contain chromophores that make them suitable for analysis by UV/Vis absorption, i. e. antibiotics, hormones, vitamins, and analgesics.

Example: Determination of the purity of aspirin tablets

- Salicylic acid, which is produced by the hydrolysis of acetylsalicylic acid, is an undesirable impurity in aspirin tablets, and should not be present at more than 0.01% w/w.
- Samples can be screened for unacceptable levels of salicylic acid by monitoring the absorbance at a wavelength of 312 nm.
- Acetylsalicylic acid absorbs at 280 nm, but absorbs poorly at 312 nm.
- Conditions for preparing the sample are chosen such that an absorbance of greater than 0.02 signifies an unacceptable level of salicylic acid.

Case study-3: Blood alcohol level Breathalyzer test

UV/Vis molecular absorption is routinely used in the analysis of narcotics and for drug testing.

- A 52.5 mL breath sample, is bubbled through an acidified solution of $K_2Cr_2O_7$.
- Ethanol present in the breath sample is oxidized by the dichromate, producing acetic acid and Cr^{3+} as products.
- The concentration of ethanol in the breath sample is determined from the decrease in absorbance at 440 nm where the dichromate ion absorbs.
- A blood alcohol content of 0.10%, which is the legal limit in most states, corresponds to 0.025 mg of ethanol in the breath sample.

What if a molecule does not absorb radiation?

If a compound does not absorb in the ultraviolet or visible region, it may be possible to prepare a derivative of it that does.

- Creatinine in blood is reacted with picrate ion in alkaline solution to form a colored product that absorbs at 490 nm.
- Uric acid is oxidized with alkaline phosphotungstate ($H_3PW_{12}O_{40}$), and the blue reduction product of hosphotungstate is measured at 680 nm.

Lower wavelength usability limit of solvents in the UV region

The cutoff point of a solvent is the wavelength below which the solvent itself absorbs most of the light (A=1)

Solvent	Cutoff Point (nm)	Solvent	Cutoff Point (nm)[a]
Water[b]	200	Dichloromethane	233
Ethanol (95%)	205	Butyl ether	235
Acetonitrile	210	Chloroform	245
Cyclohexane	210	Ethyl proprionate	255
Cyclopentane	210	Methyl formate	260
Heptane	210	Carbon tetrachloride	265
Hexane	210	*N*, *N*-Dimethylformamide	270
Methanol	210	Benzene	280
Pentane	210	Toluene	285
Isopropyl alcohol	210	*m*-Xylene	290
Isooctane	215	Pyridine	305
Dioxane	220	Acetone	330
Diethyl ether	220	Bromoform	360
Glycerol	220	Carbon disulfide	380
1,2-Dichloroethane	230	Nitromethane	380

Deviation from Beer's Law

✓ Chemical deviations

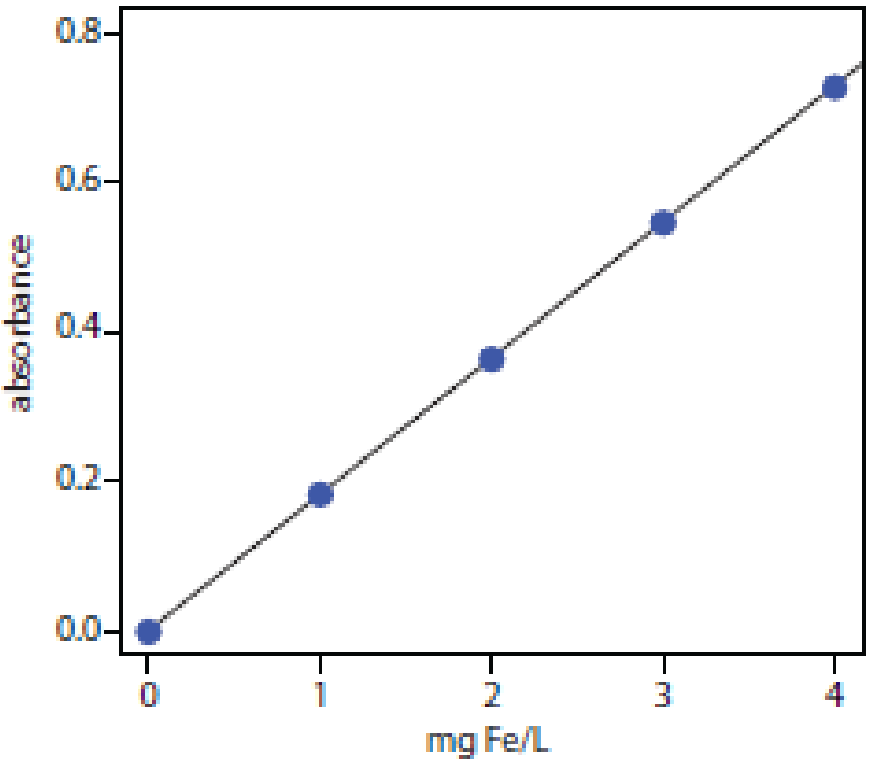
1. Chemical causes for nonlinearity occur when an asymmetric chemical equilibrium exists. Ex. weak acid that absorbs at a particular wavelength but has an anion that does not.
2. colored (absorbing) metal ion complexes or chelates in the absence of a large excess of the complexing agent.

✓ Instrumental deviations

1. Stray radiation 2. Nonlinearity 3. Nonuniform cell thickness.

Other chemical and instrumental sources of nonlinearity in absorbance measurements may include: hydrogen bonding, interaction with the solvent, nonlinear detector response or nonlinear electronic amplification, noncollimated radiation, and signal saturation.

Calculation Example



mg Fe/L	absorbance
0.00	0.000
1.00	0.183
2.00	0.364
3.00	0.546
4.00	0.727
sample	0.269

A=0.0006+0.1817×C(mgFe/L)

Infrared Absorption and Molecular Structure

- Infrared spectroscopy (IR spectroscopy) involves the interaction of infrared radiation with matter.
- IR spectroscopy can be used to identify and study chemical substances.
- Samples may be solid, liquid, or gas.
- The infrared spectrum is divided into three regions:

✓Near-IR: 14000–4000 cm^{-1} (0.7–2.5 μm wavelength),

✓Mid-IR: 4000–400 cm^{-1} (2.5–25 μm),

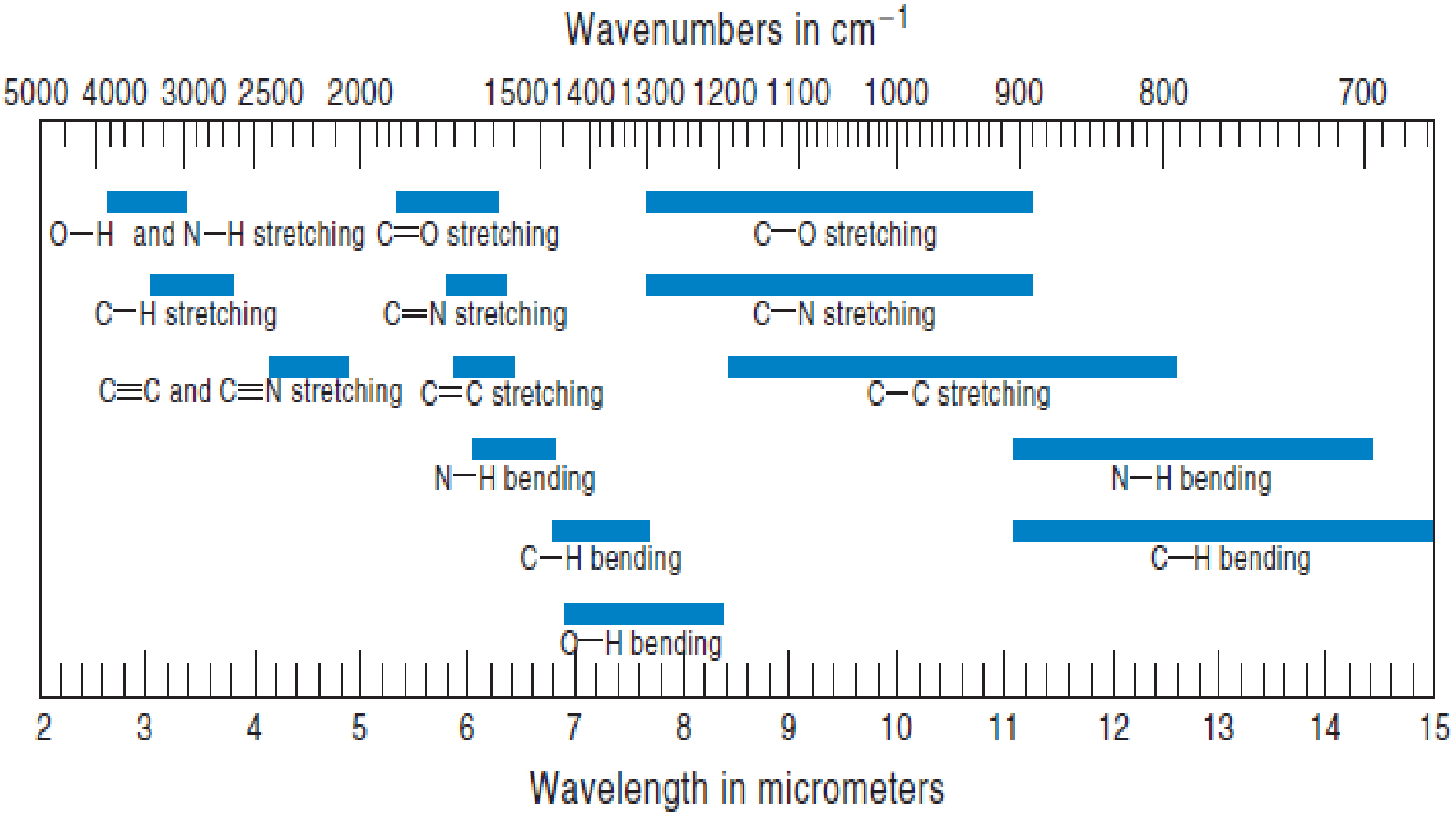
✓Far-IR: 400–10 cm^{-1} (25–1000 μm).

Number of vibrational modes: Vibrational degrees of freedom

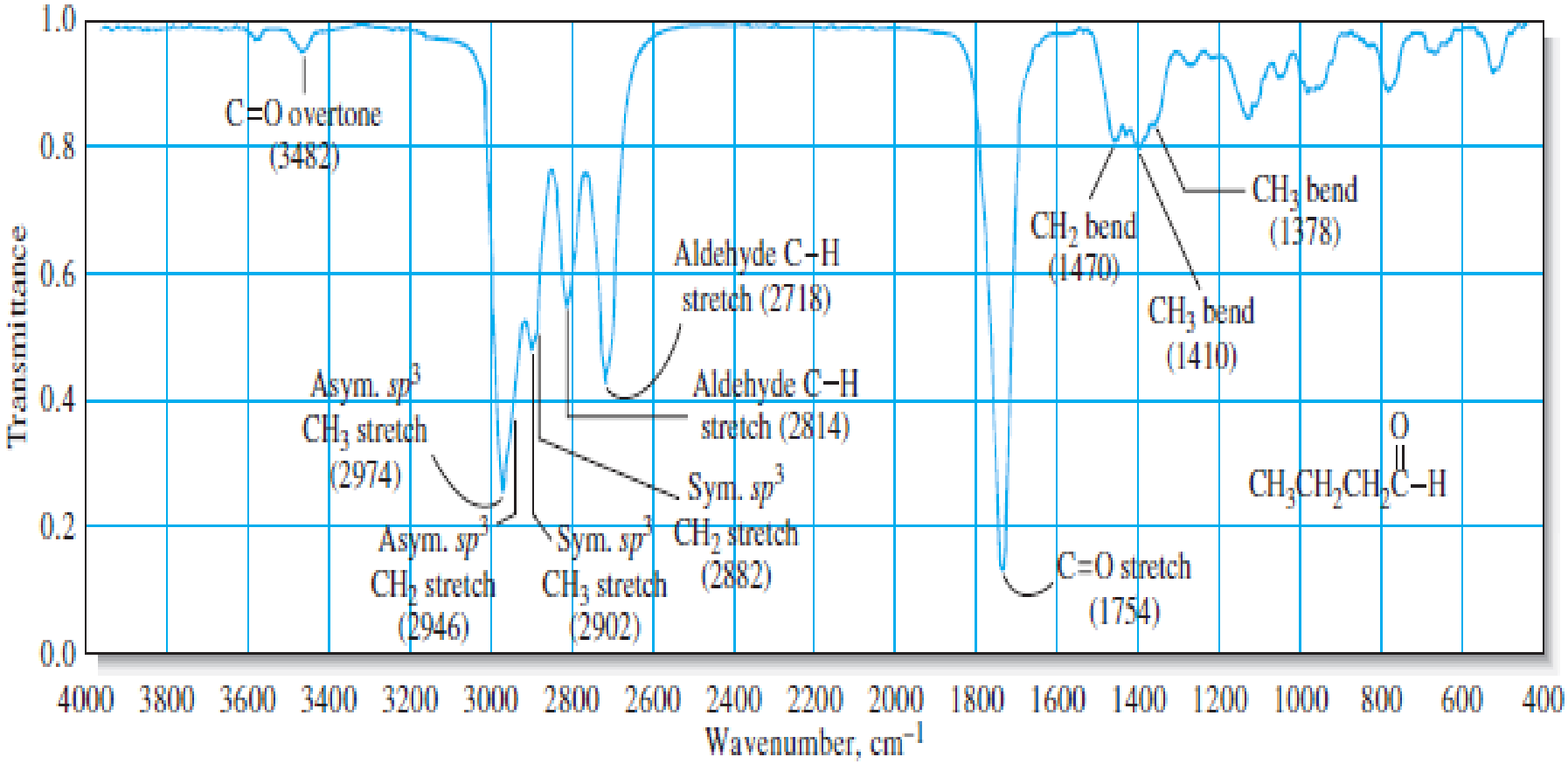
Molecules with N number of atoms:

- Linear molecules have (3N–5) degrees of vibrational modes,
- Non-linear molecules have (3N–6) degrees of vibrational modes. Example: H_2O, non-linear molecule, will have $3\times3–6=3$ degrees of vibrational freedom. Benzene (C_6H_6), (30 V. modes).
- Simple diatomic molecules have only one bond and only one vibrational band.
- Symmetrical molecule, N_2, not observed in the IR spectrum.
- Asymmetrical diatomic molecules, e.g. CO, absorb in the IR spectrum.
- Complex molecules give complex IR spectra (many peaks).

Group vibrations and Regions of IR absorption



Group vibrations and Regions of IR absorption

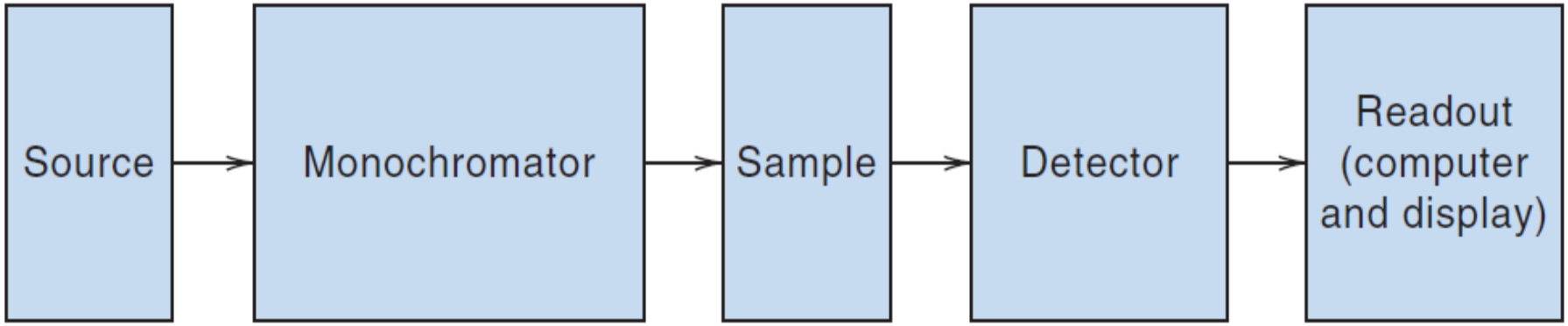


Group vibrations and Regions of IR absorption

Some Characteristic Infrared Absorption Peaks

	Functional Group	Absorption Peaks	
		Wavenumber, cm^{-1}	Wavelength, μm
O—H	Aliphatic and aromatic	3600–3000	2.8–3.3
NH_2	Also secondary and tertiary	3600–3100	2.8–3.2
C—H	Aromatic	3150–3000	3.2–3.3
C—H	Aliphatic	3000–2850	3.3–3.5
C≡N	Nitrile	2400–2200	4.2–4.6
C≡C—	Alkyne	2260–2100	4.4–4.8
COOR	Ester	1750–1700	5.7–5.9
COOH	Carboxylic acid	1740–1670	5.7–6.0
C=O	Aldehydes and ketones	1740–1660	5.7–6.0
$CONH_2$	Amides	1720–1640	5.8–6.1
C=C—	Alkene	1670–1610	6.0–6.2
ϕ—O—R	Aromatic	1300–1180	7.7–8.5
R—O—R	Aliphatic	1160–1060	8.6–9.4

Common Sources of Electromagnetic Radiation for Spectroscopy



Source	Wavelength Region	Useful for
H_2 and D_2 lamp	continuum source from 160–380 nm	UV molecular absorption
tungsten lamp	continuum source from 320–2400 nm	Vis molecular absorption
Xe arc lamp	continuum source from 200–1000 nm	molecular fluorescence
Nernst glower	continuum source from 0.4–20 μm	IR molecular absorption
globar	continuum source from 1–40 μm	IR molecular absorption
nichrome wire	continuum source from 0.75–20 μm	IR molecular absorption
hollow cathode lamp	line source in UV/Vis	atomic absorption
Hg vapor lamp	line source in UV/Vis	molecular fluorescence
laser	line source in UV/Vis	atomic and molecular absorption, fluorescence and scattering

Common Sources of Electromagnetic Radiation for Spectroscopy

Source	Wavelength Region	Useful for
H_2 and D_2 lamp	continuum source from 160–380 nm	UV molecular absorption
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Nernst glower	continuum source from 0.4–20 μm	IR molecular absorption
globar	continuum source from 1–40 μm	IR molecular absorption
nichrome wire	continuum source from 0.75–20 μm	IR molecular absorption
hollow cathode lamp	line source in UV/Vis	atomic absorption
Hg vapor lamp	line source in UV/Vis	molecular fluorescence
laser	line source in UV/Vis	atomic and molecular absorption, fluorescence and scattering

Xe arc Lamp

Nernst Glower

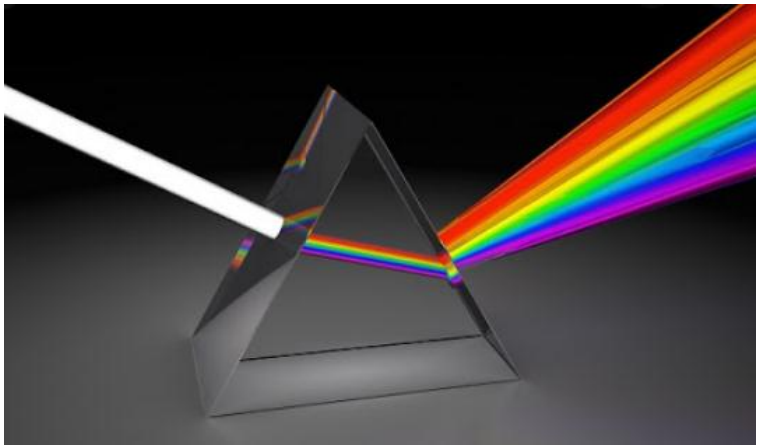
D_2 Lamp

H_2 Lamp

Monochromators

Monochromator: an optical device transmits a mechanically selectable narrow band of wavelengths of light or other radiation, from a wider range of wavelengths available at the input.

Diffraction Gratings



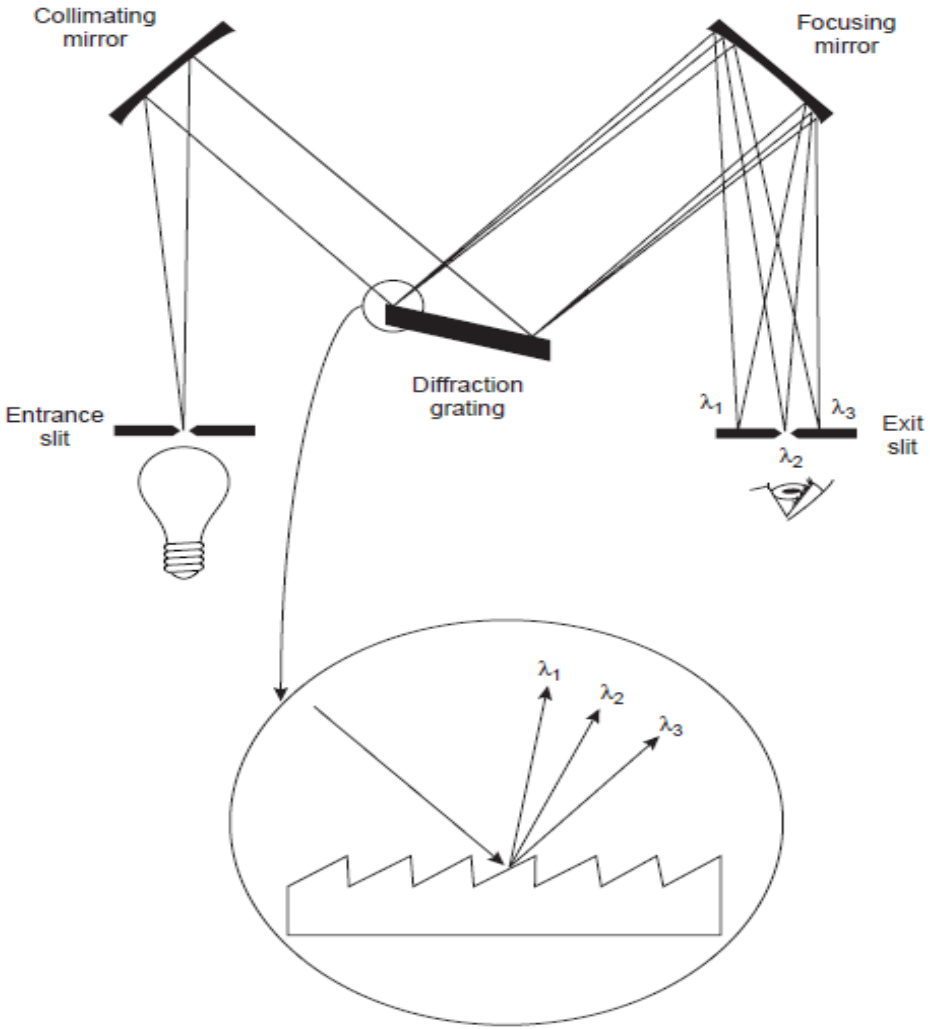
Prisms

Optical Filters

Collimator

A **collimator** is a device which narrows a beam of particles or waves.

- To cause the directions of motion to become more aligned in a specific direction,
- To cause the spatial cross section of the beam to become smaller.



A Polychromatic source to a **Monochromatic** source

Detectors

- **Detectors:**

❖ UV-Vis

1.Phototube: Phototubes operate according to the photoelectric effect: Incoming photons strike a photocathode, knocking electrons out of its surface, which are attracted to an anode. Thus current is dependent on the frequency and intensity of incoming photons.

2.Photomultiplier tube (PM tube): more sensitive than a phototube, these detectors multiply the current produced by incident light by as much as 100 million times.

3.silicon diode array,

4. The charge coupled device photosensor (CCD) array,

Detectors

- **Detectors:**

❖ **IR**

Infrared detectors fall in two categories, one relies on the quantum properties of EMR and the other relies on the fact that infrared radiation is essentially heat and these detectors are heat sensors.

1.Thermocouples,

2.bolometers,

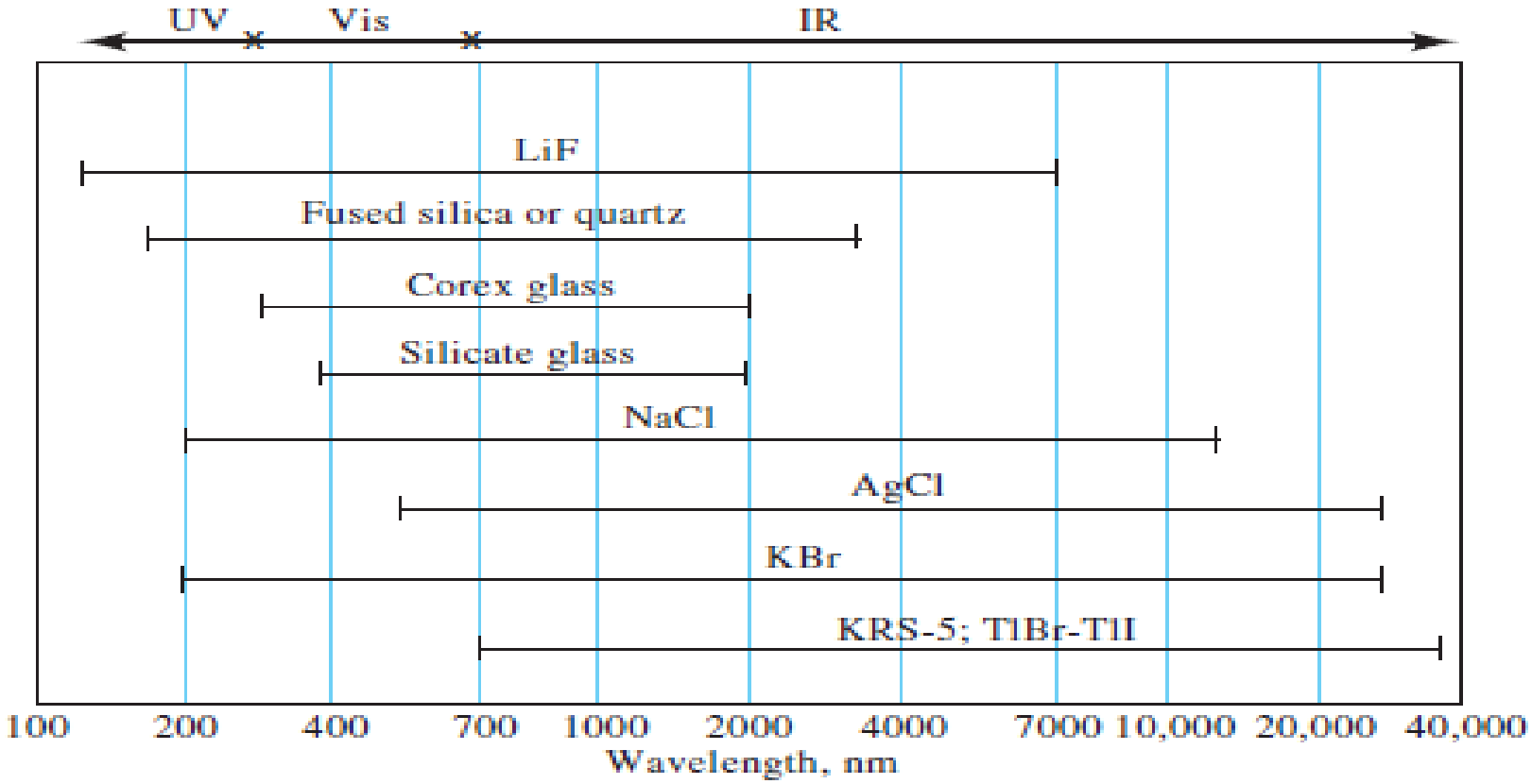
3. thermistors,

4.InGaAs diode array

Sample Cells

Optical Materials

UV : (quartz) Vis : (glass, quartz) IR : (salt crystals)



Spectrophotometers

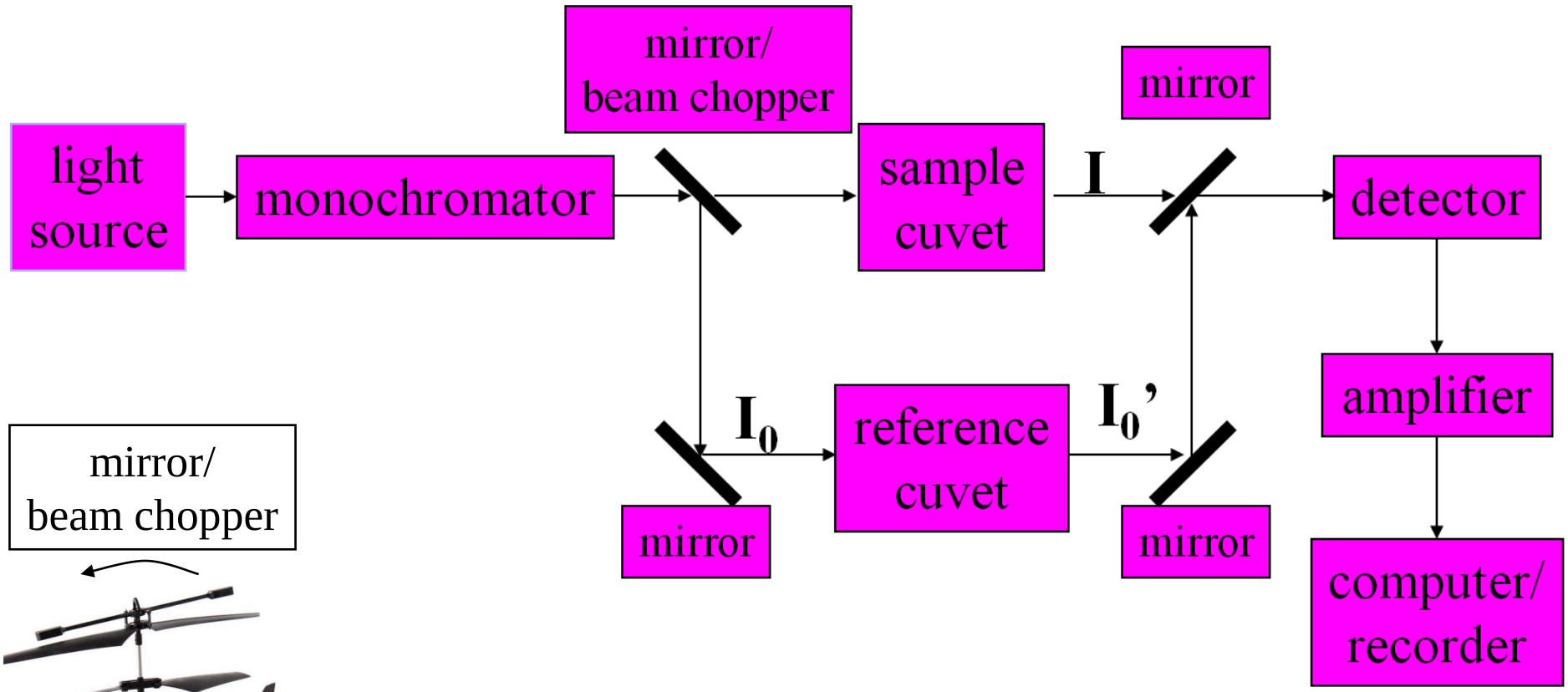
1. Single-beam spectrophotometer
   – insert reference blank once at beginning of exp't
   – only measure absorbance of EITHER sample OR reference blank at a time.
2. Double-beam spectrophotometer
   – continuously checks reference blank to account for changes in
     - source intensity (I_0)
     - detector response

if absorbance measurement from reference blank changes, spectrophotometer corrects for that change to find true absorbance of analyte.

The simplest instrument for IR absorption spectroscopy is a filter photometer similar to that for UV/Vis absorption.

Double-beam Scanning Spectrophotometer

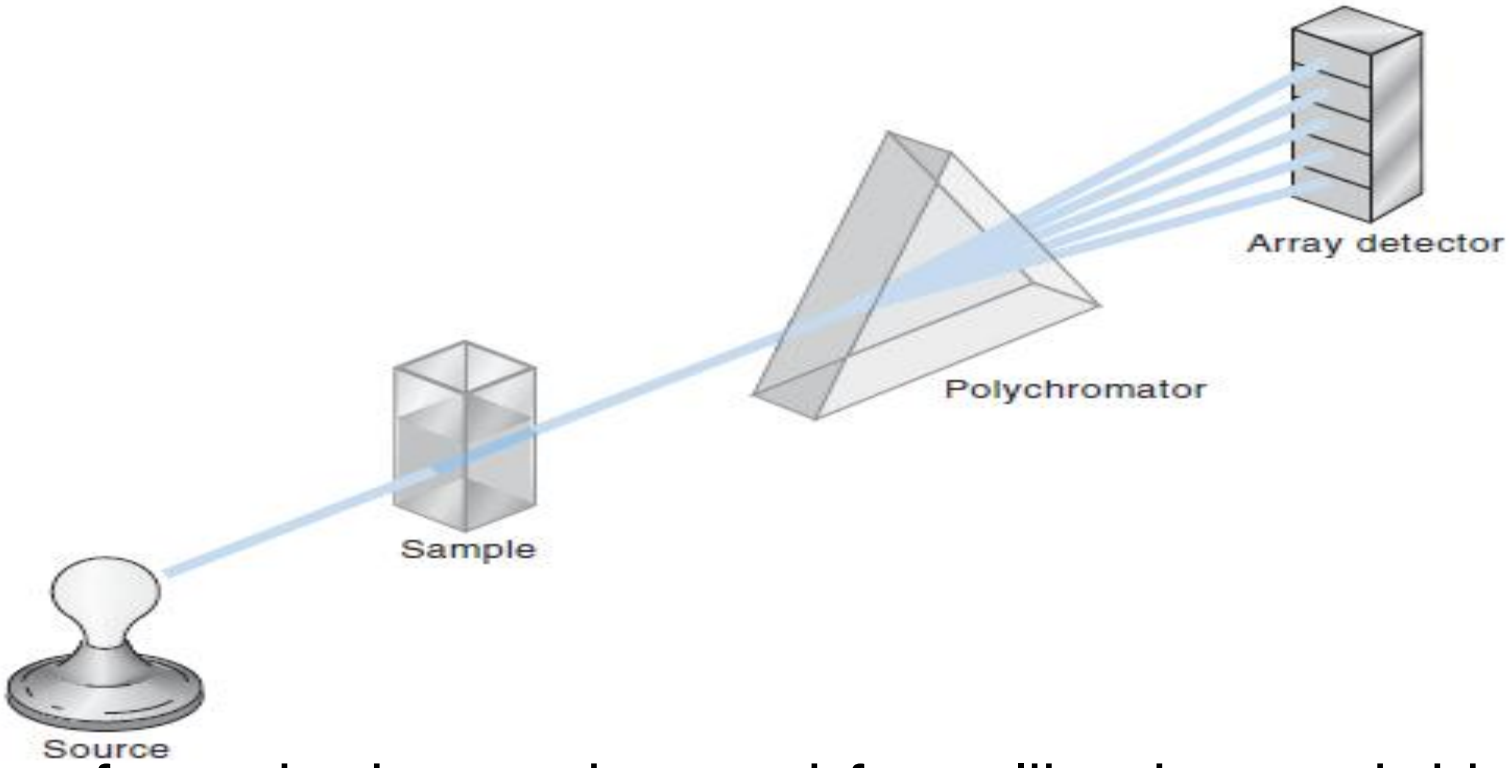
- Alternately measuring I_0 and I by diverting light beam through reference cuvet



Array Spectrometers

3. Array Spectrometers—Getting the Entire Spectrum at Once.

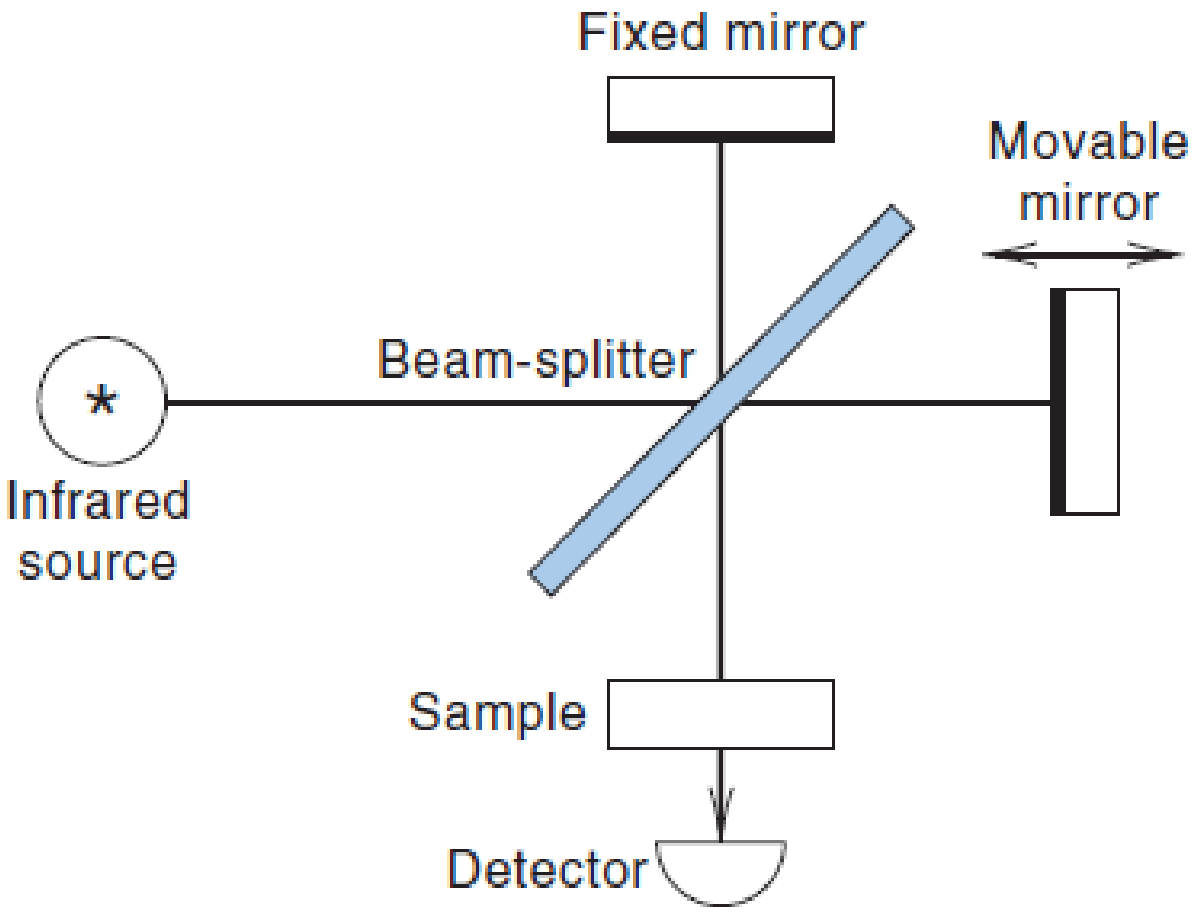
The absorbance at many points can be simultaneously measured



Mixtures of standards may be used for calibration, and this can compensate for possible interactions between components.

FTIR spectrometry

Fourier Transform Infrared Spectrometers

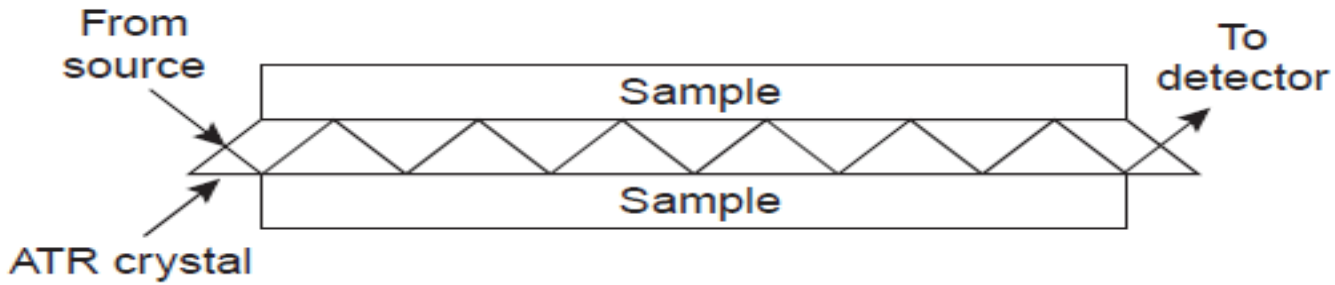


IR Spectrometer

- IR analysis of liquid samples??? Salt cuvet!!!

The analysis of aqueous solutions is complicated by the solubility of the NaCl cell window in water.

The Attenuated Total Reflectance (ATR) instead of transmission.

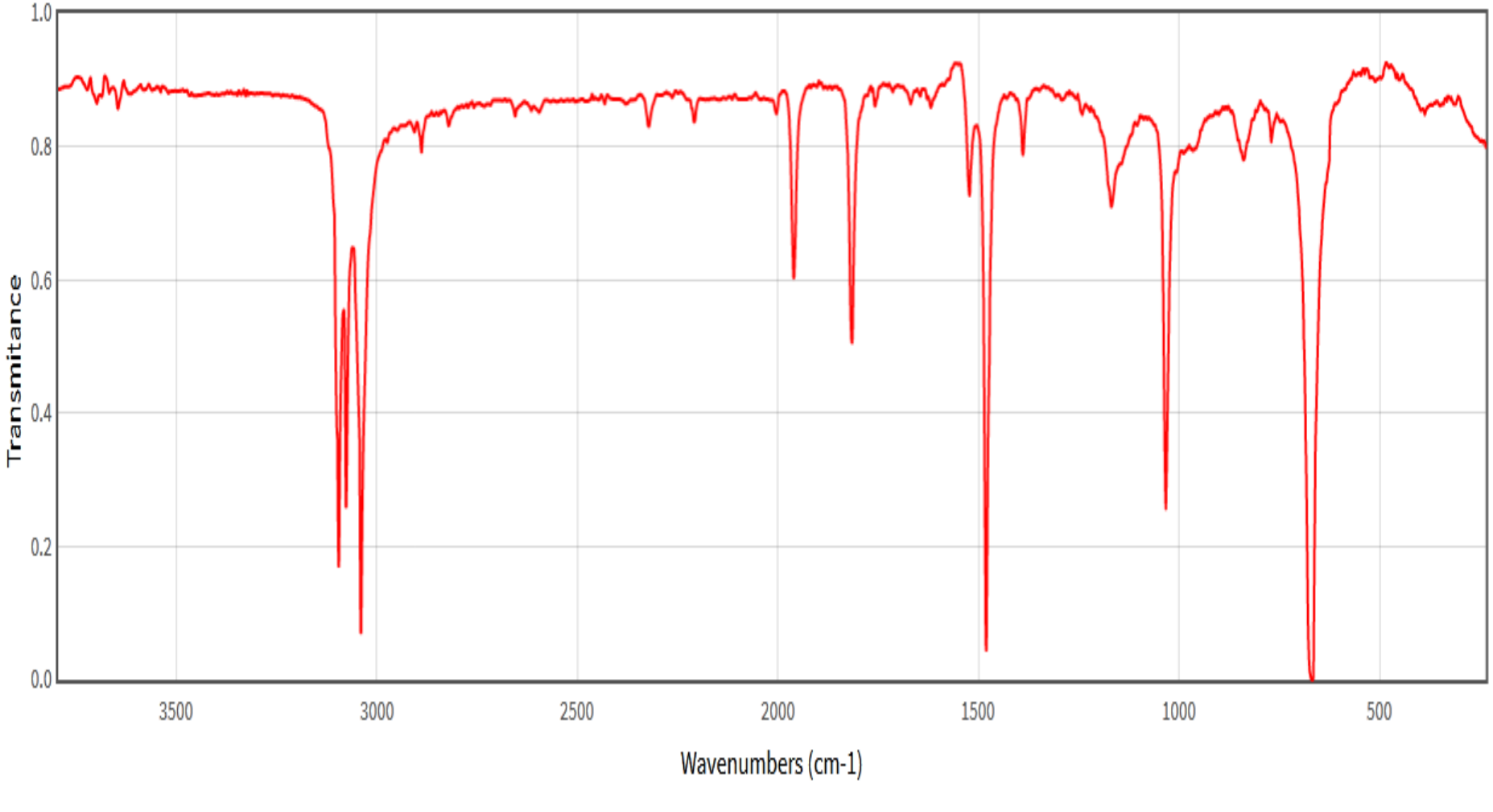


ATR sampler, consisting of an IR-transparent crystal of high refractive index, such as ZnSe, surrounded by a sample of lower-refractive index.

Refractive index=2.38 for Diamond, 2.61 for ZnSe, 3.68 for Silicon.

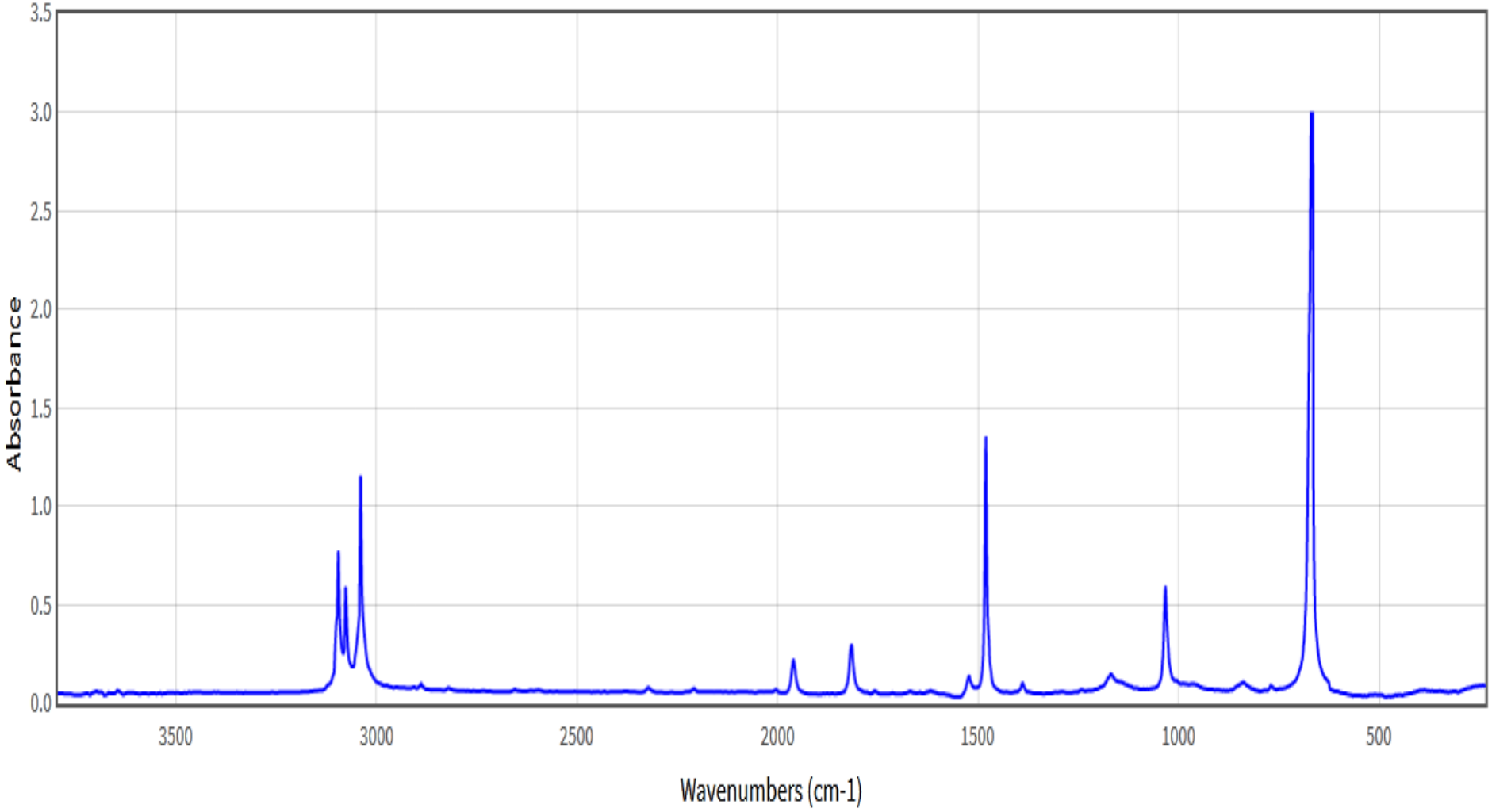
IR spectra: Be careful

Benzene Infrared Spectrum



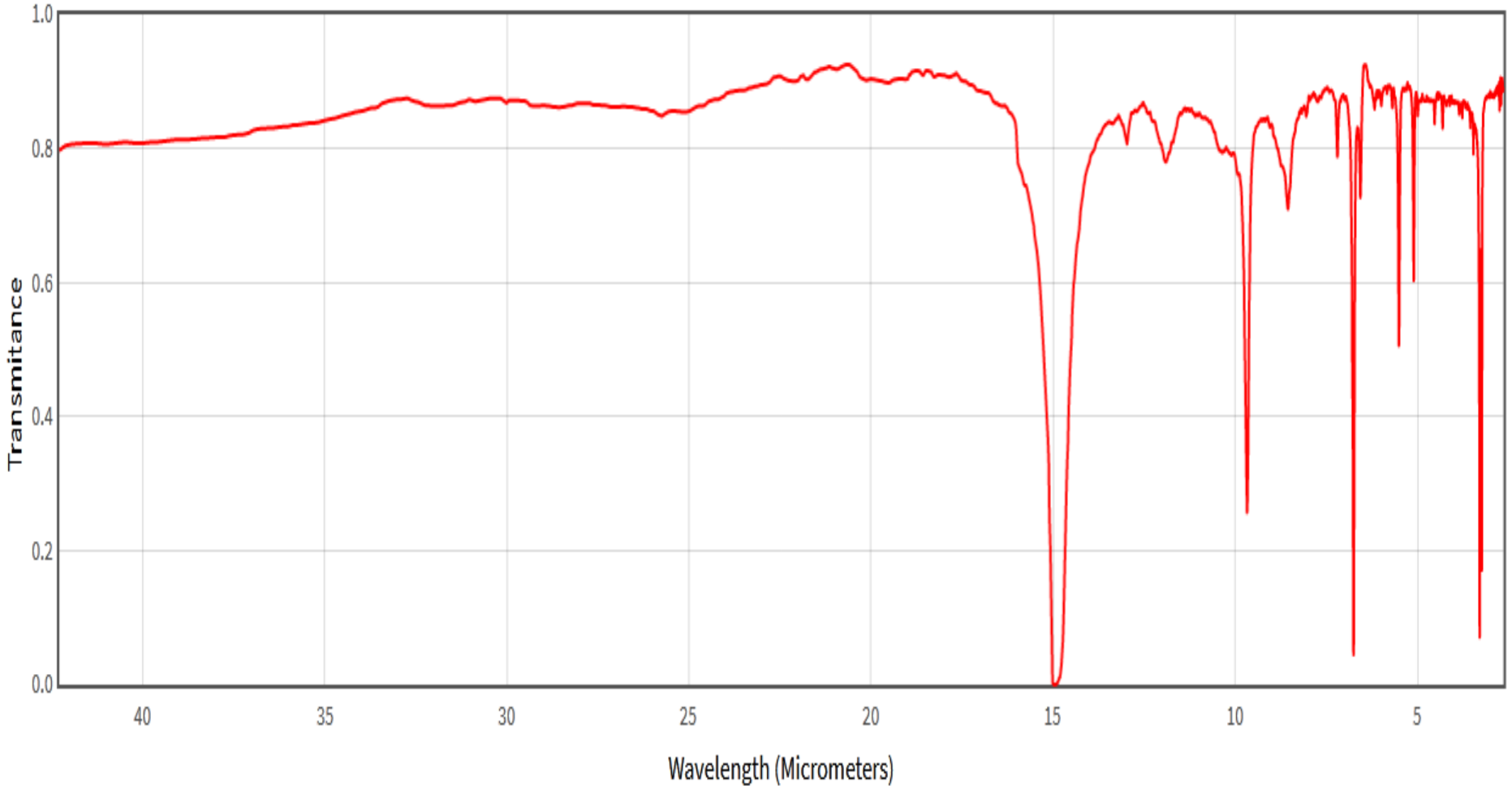
IR spectra: Be careful

Benzene Infrared Spectrum



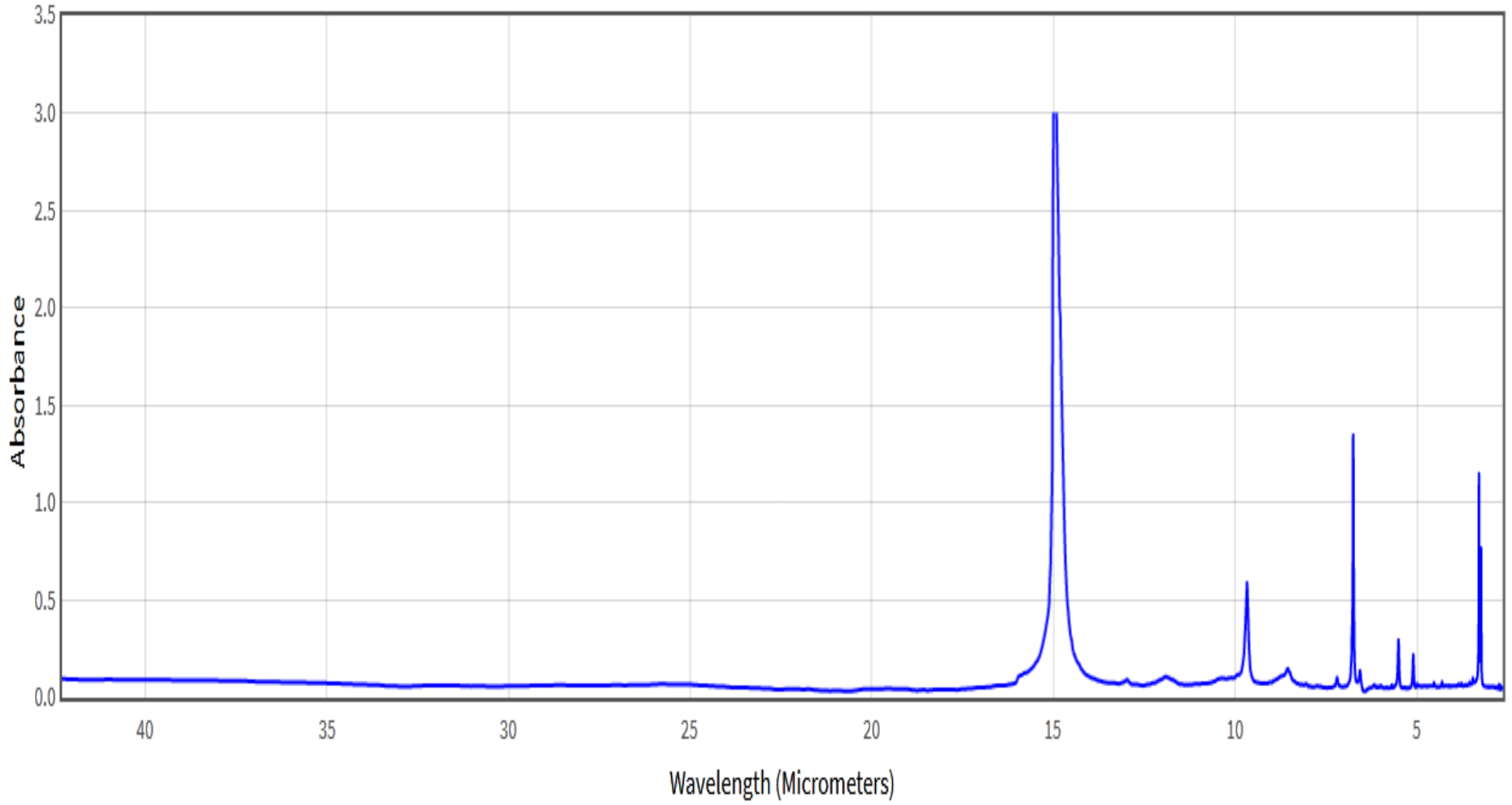
IR spectra: Be careful

Benzene Infrared Spectrum



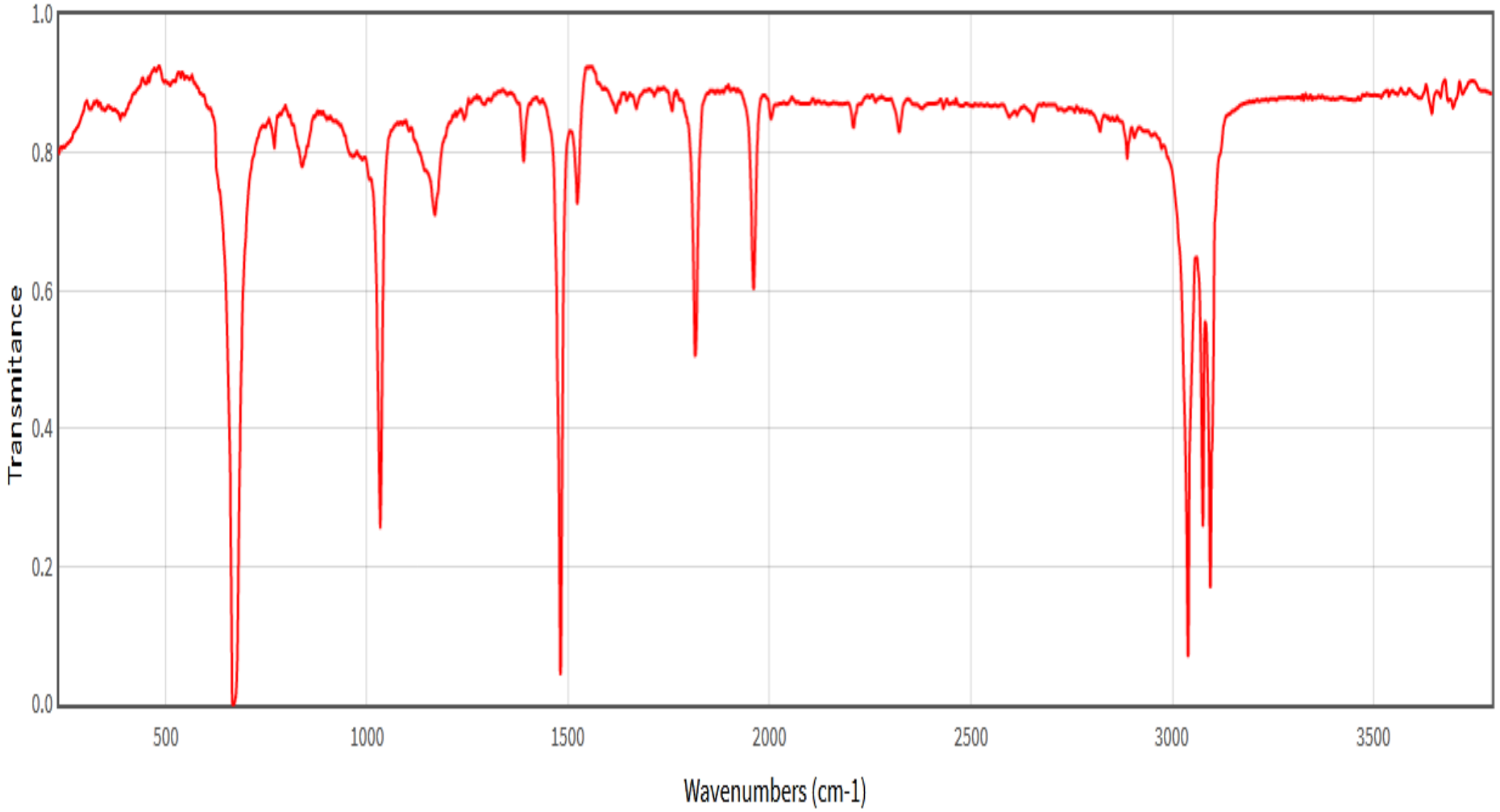
IR spectra: Be careful

Benzene Infrared Spectrum

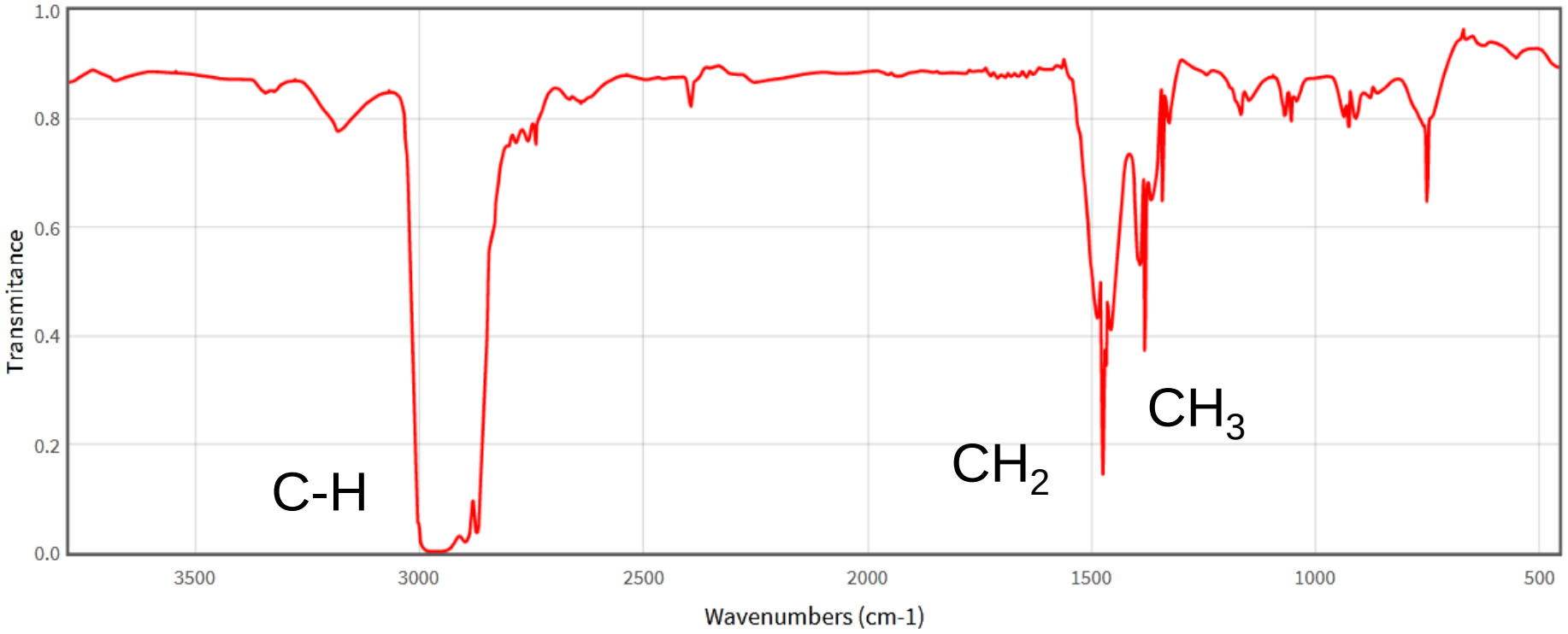


IR spectra: Be careful

Benzene Infrared Spectrum



IR Spectrum Interpretation



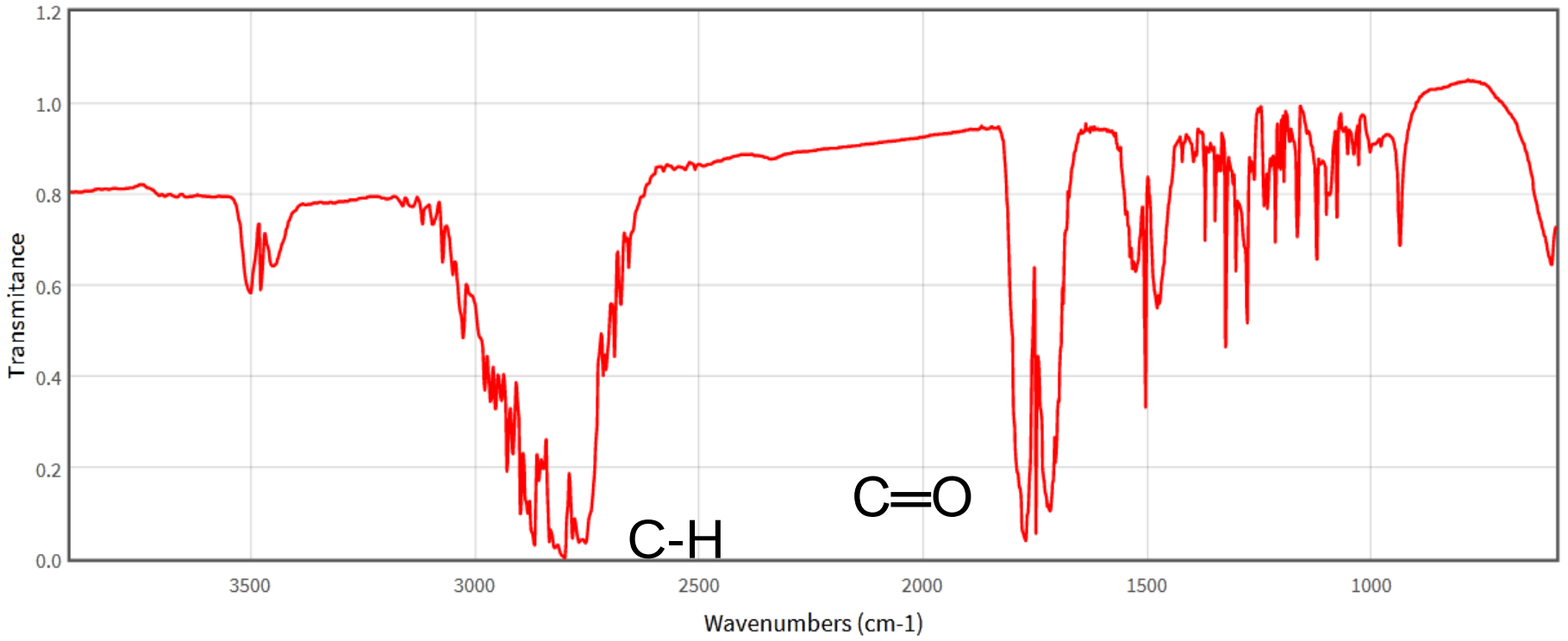
$$\boldsymbol{CH_3 - CH_2 - CH_3}$$

CH_3: 1375 cm^{-1}

CH_2: 1450 cm^{-1}

C-H: 3000 cm^{-1} and 1375 cm^{-1} and 1450 cm^{-1}

IR Spectrum Interpretation

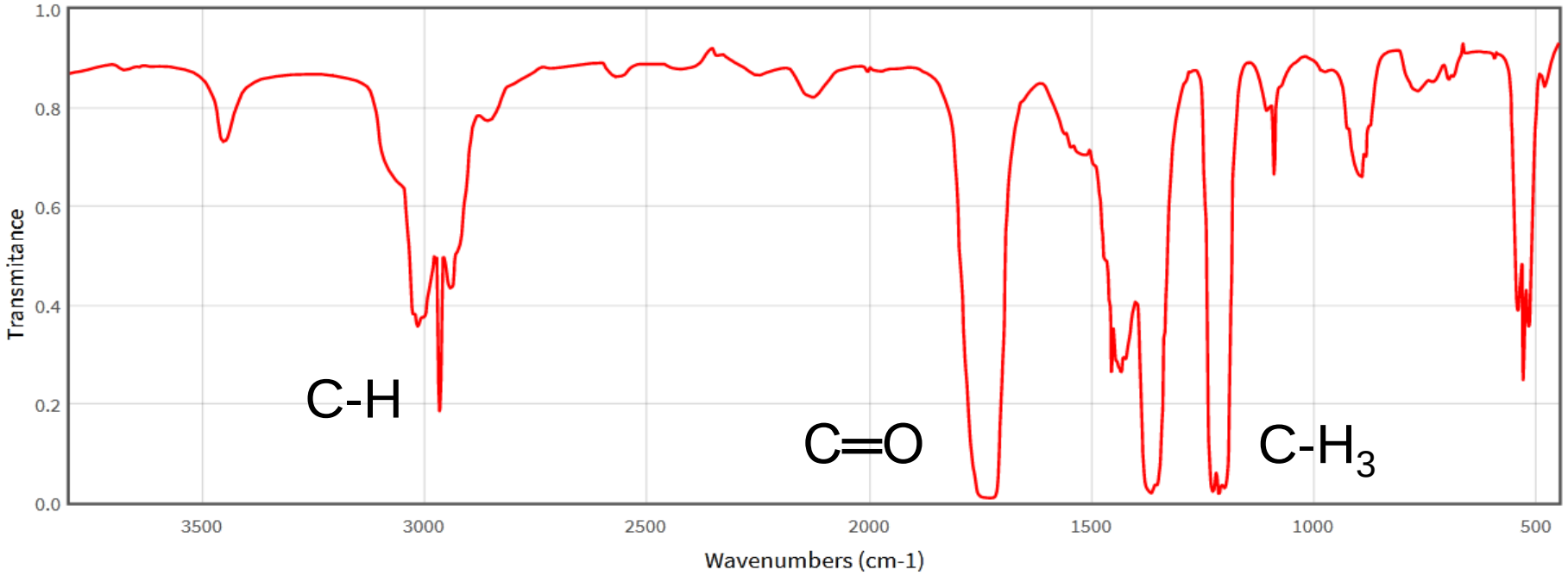


$$\boldsymbol{H - CHO}$$

C=O: 1660-1820 cm^{-1}

C-H (Aldehyde): 2750-2850 cm^{-1}

IR Spectrum Interpretation



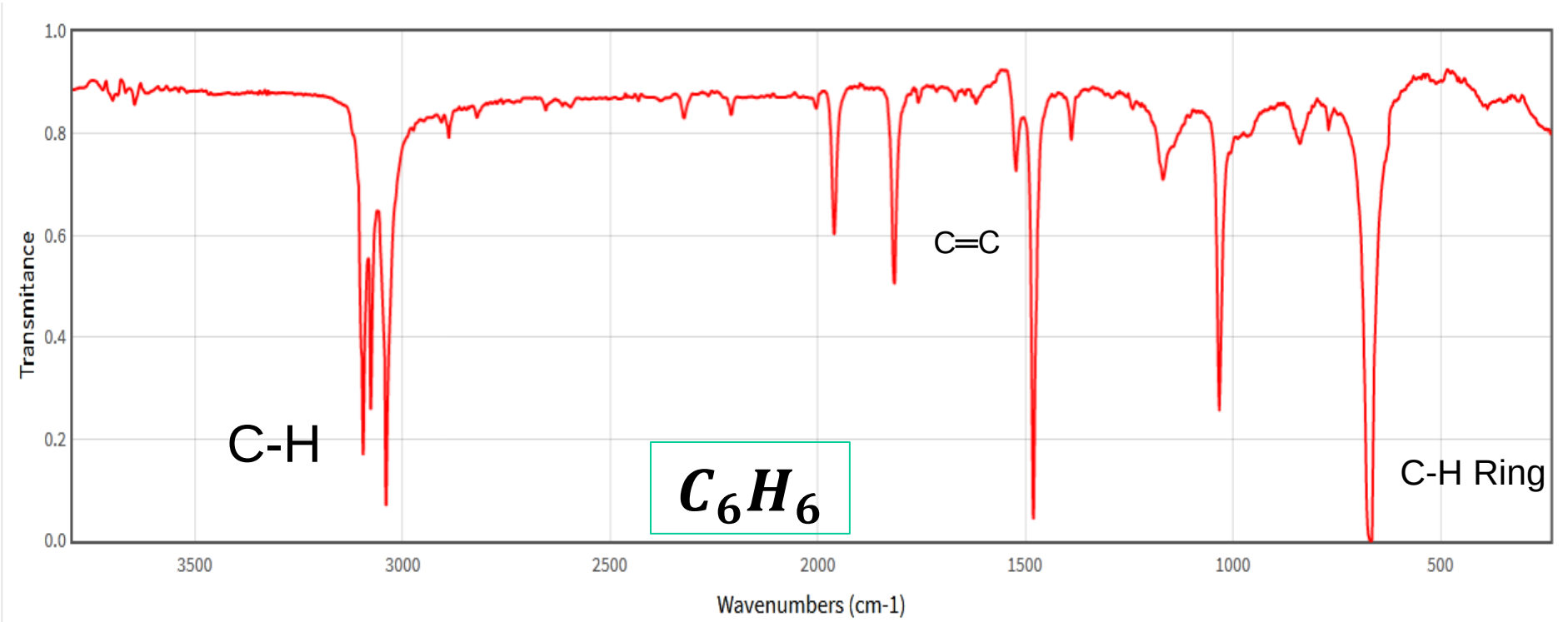
$\boldsymbol{CH_3 - CO - CH_3}$

C=O: 1660-1820 cm^{-1}

CH_3: 1375 cm^{-1}

C-H: 3000 cm^{-1} and 1375 cm^{-1} and 1450 cm^{-1}

IR Spectrum Interpretation



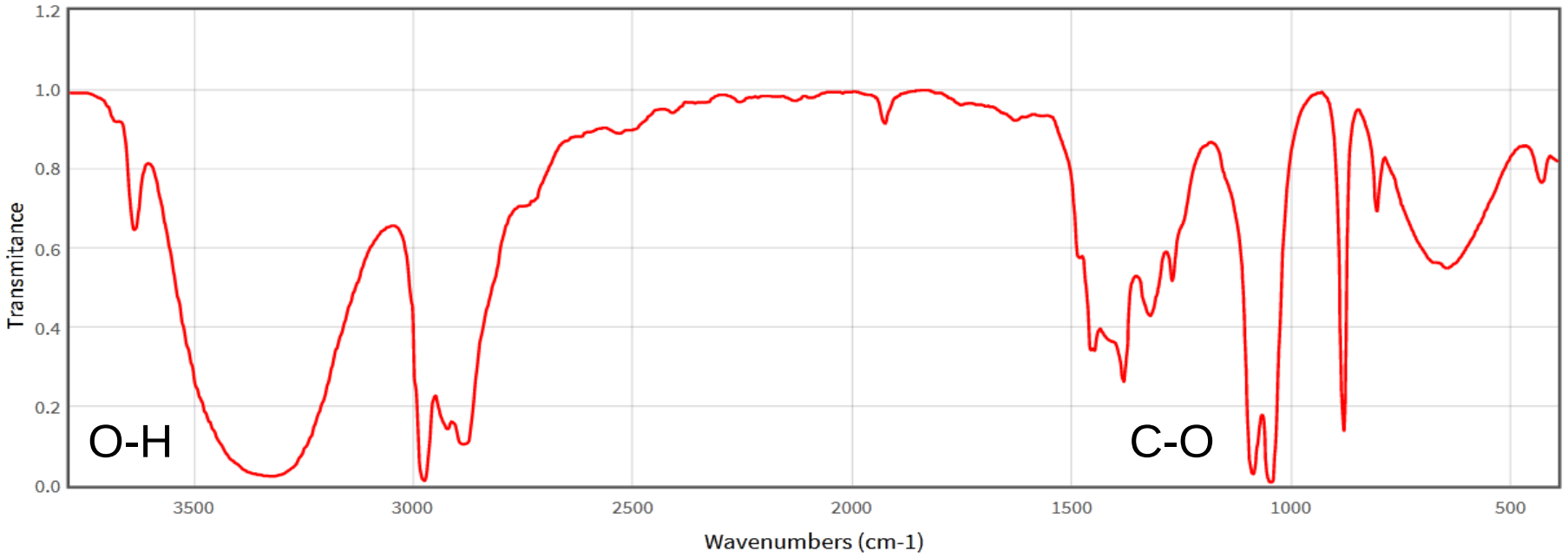
C=C: 1650 cm^{-1} (Weak),

C-H (Aromatic): left of 3000 cm^{-1},

Moderate to strong (1450-1650 cm^{-1}): Aromatic Ring.

Ring: 750 cm^{-1} (It can be shifted to 800 or 850 for meta or para rings),

IR Spectrum Interpretation



$$CH_3 - CH_2 - OH$$

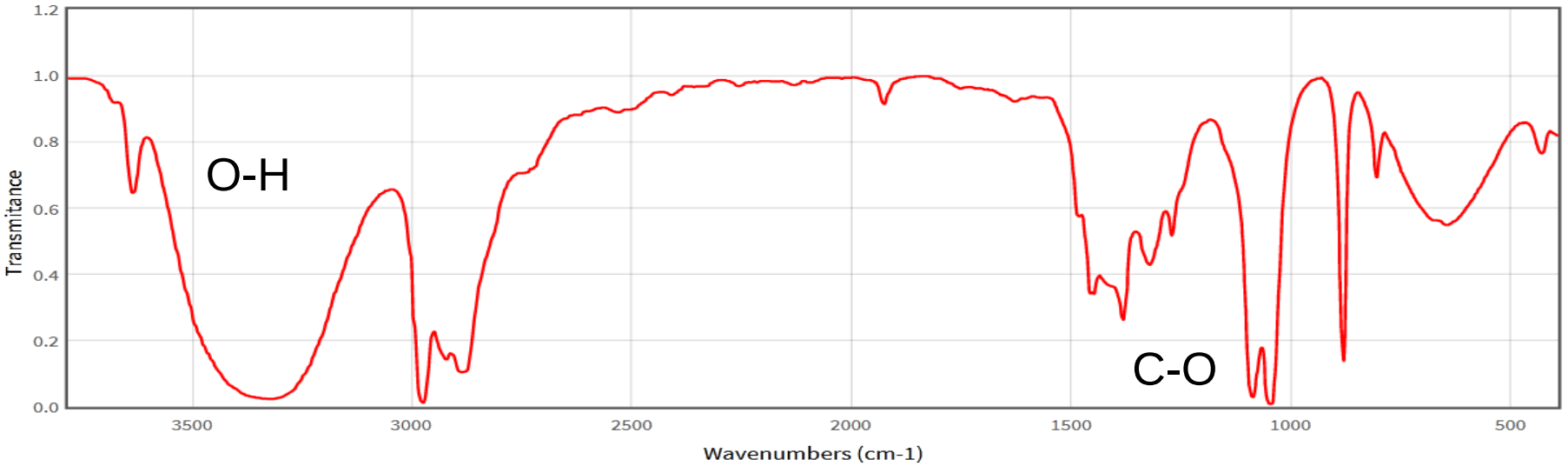
O-H: 3300-3600 cm^{-1} Wide Band,

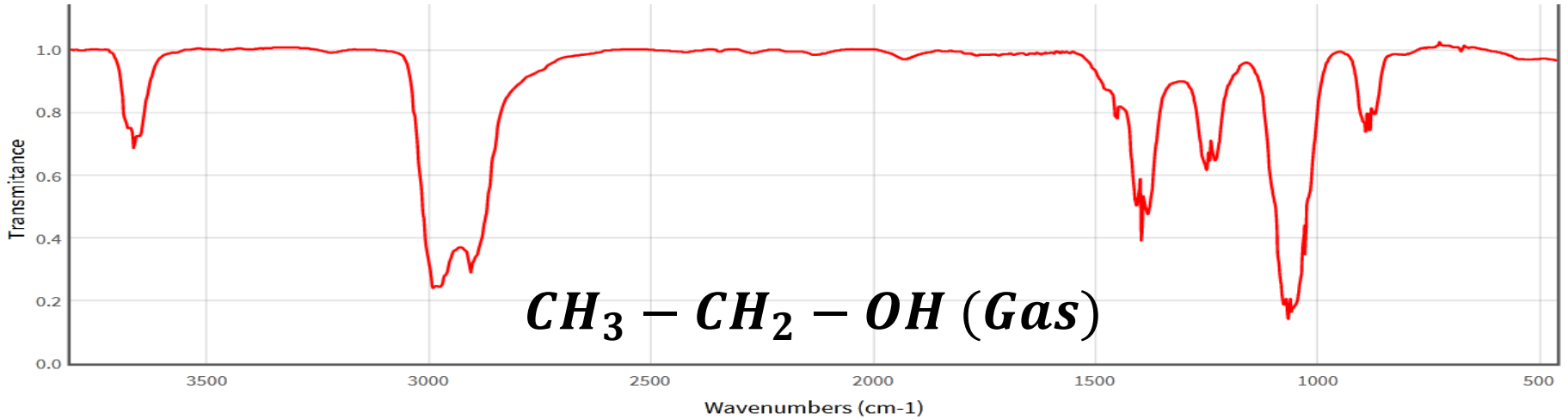
C-O: 1000-1300 cm^{-1}

IR Spectrum Interpretation

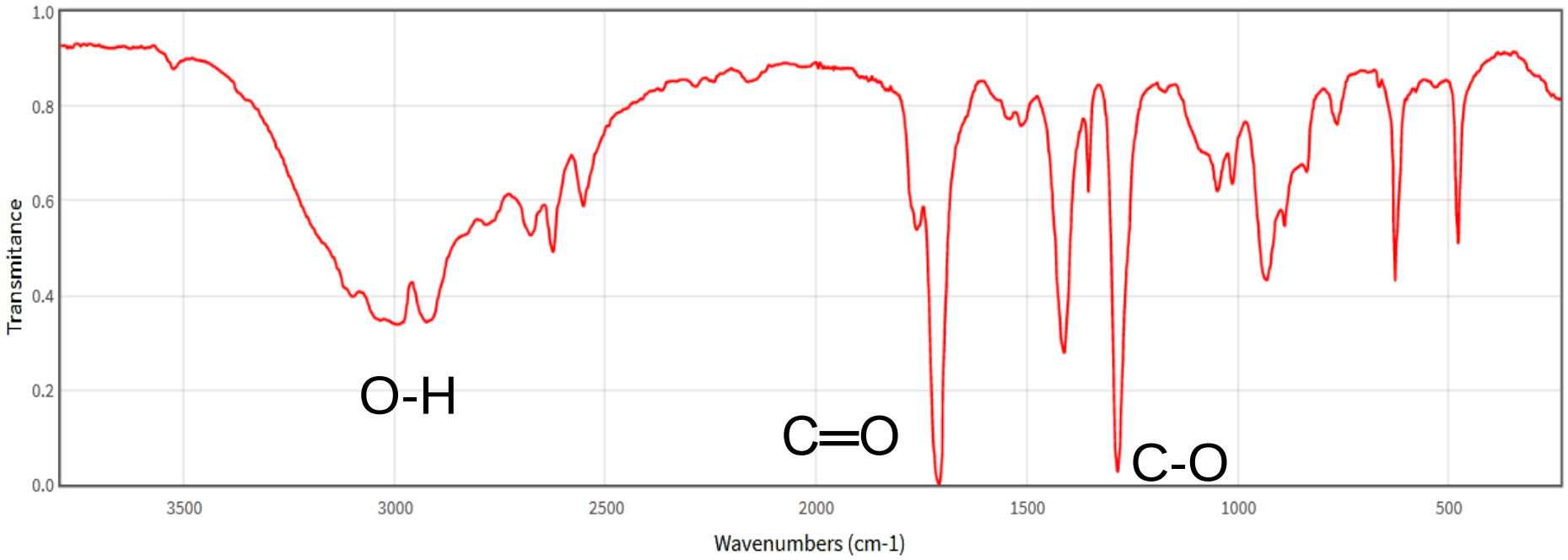
$$CH_3 - CH_2 - OH\ (Liquid)$$

O-H: 3300-3600 cm^{-1} Wide Band, **C-O**: 1000-1300 cm^{-1}





IR Spectrum Interpretation



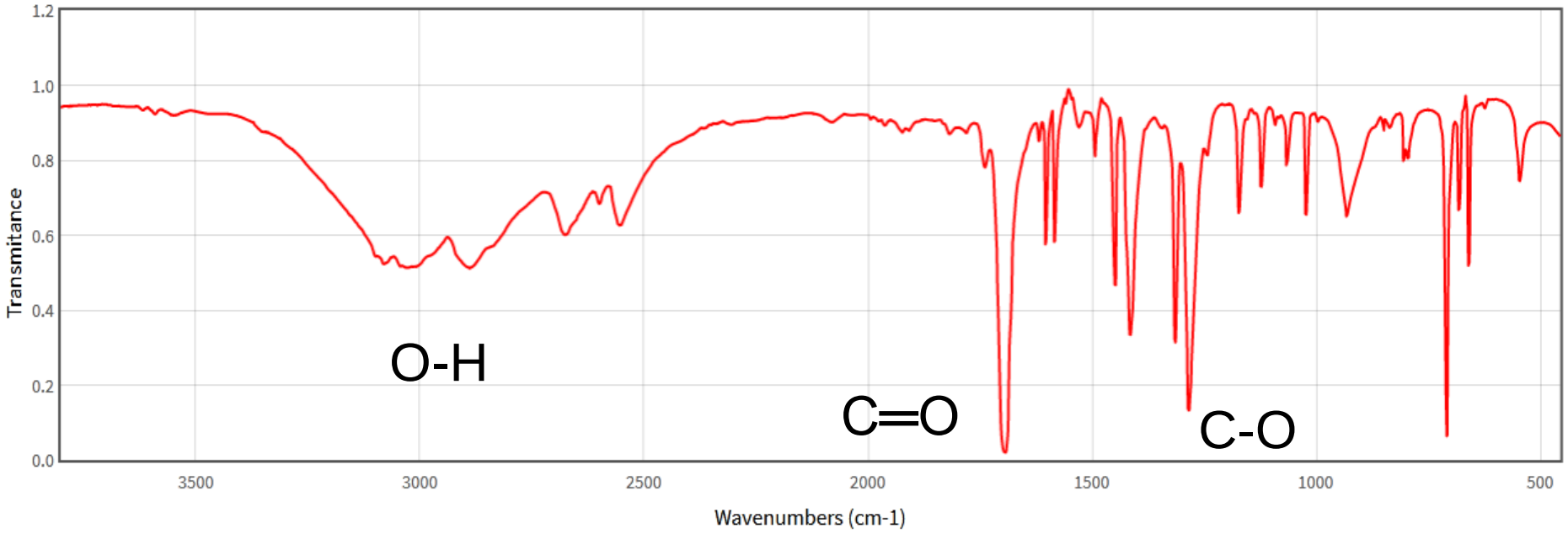
$$CH_3 - COOH$$

O-H: 2400-3400 cm^{-1} VERY Wide Band,

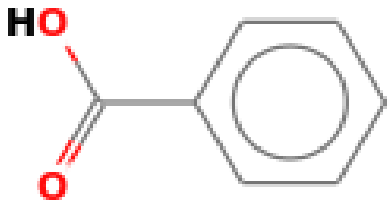
C=O: 1700-1730 cm^{-1},

C-O: 1210-1320 cm^{-1},

IR Spectrum Interpretation



$$C_6H_5 - COOH$$



O-H: 2400-3400 cm^{-1} VERY Wide Band,

C=O: 1700-1730 cm^{-1},

C-O: 1210-1320 cm^{-1},

Moderate to strong (1450-1650 cm^{-1}): Aromatic Ring.

How Can We Interpret a spectrum-1

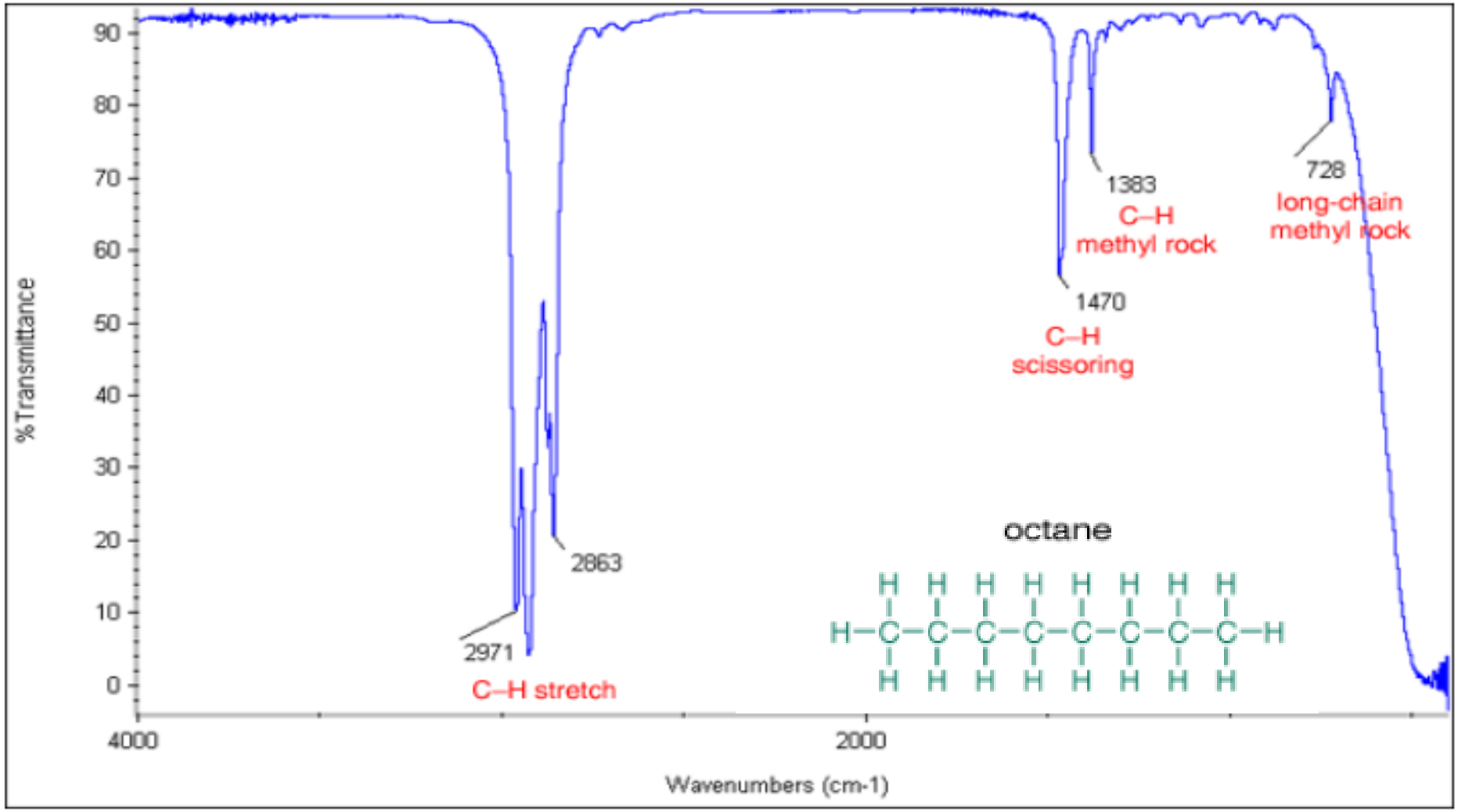
1660-1820 cm^{-1}	C=O stretch	Yes	
2400-3400 cm^{-1}	O–H stretch	Carboxylic group	Very Wide
3500 cm^{-1}	N–H stretch	Amides	
1000-1300 cm^{-1}	C-O stretch	Esters	
2750 and 2850 cm^{-1}	C-H stretch	Aldehydes	
All previous cases are not present		Ketones	

C=O: 1660-1820 cm^{-1}, (In most cases it is the longest one in the spectrum),

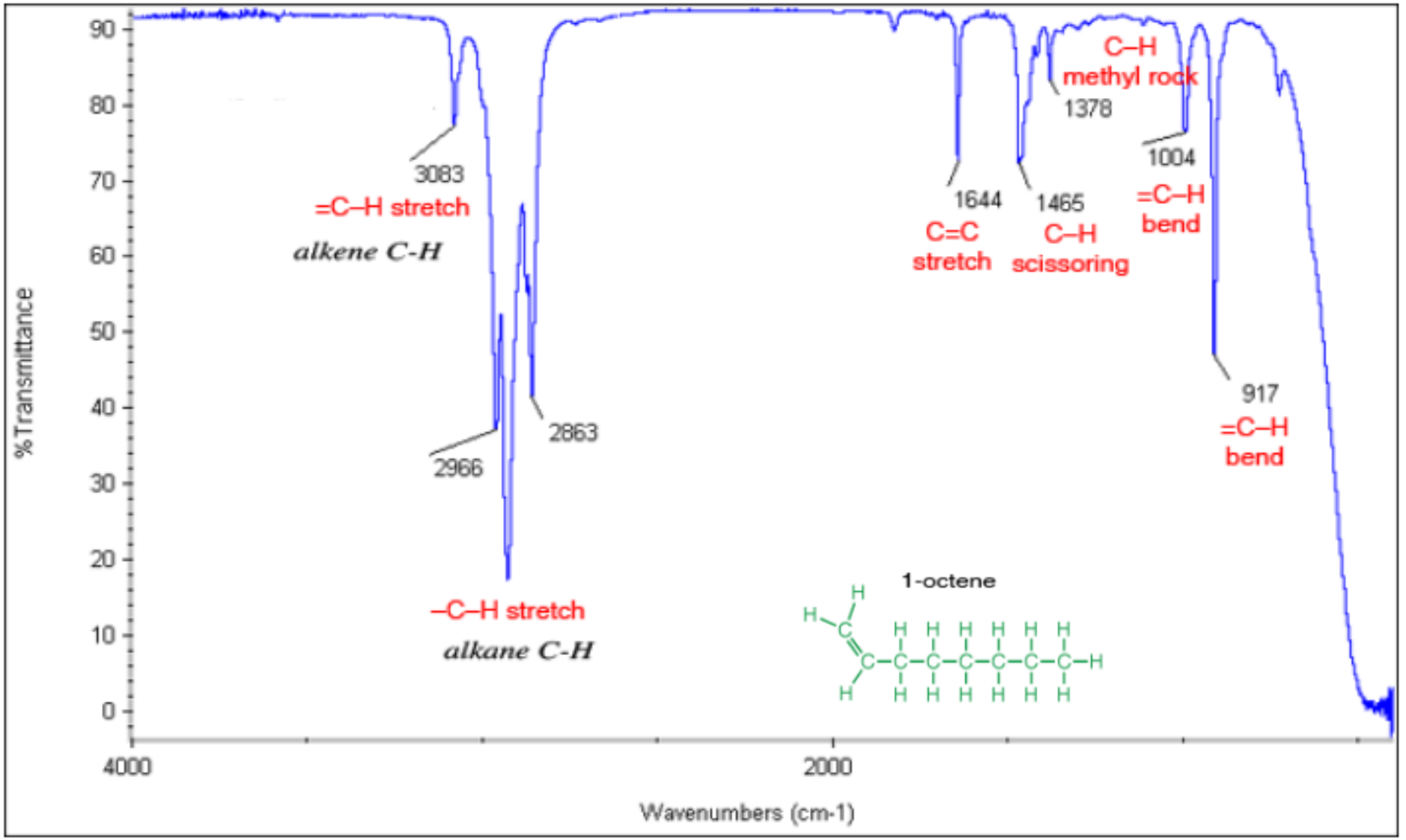
How Can We Interpret a spectrum-2

1660-1820 cm^{-1}	**C=O stretch**	**No**	
3300-3600 cm^{-1}	**O–H stretch**	Alcohols	a broad, strong band
3500 cm^{-1}	**N–H stretch**	Amines	
1000-1300 cm^{-1}	**C–O stretch**	Ethers	Verifiy that O-H is not present
1650 cm^{-1}, and 1450-1650 cm^{-1}	**C=C Aromatic**	Aromatic Ring	Weak
2150 cm^{-1}	**C≡C**	Triple bonds	Verify that C-H at 3300 cm^{-1} is present
2250 cm^{-1}	**C≡N**	Triple bonds	
1500-1600 cm^{-1}, and 1300-1390 cm^{-1}	**N-O**	Nitro group (NO_2)	
2850-3000 cm^{-1}	**C–H stretch**	Alkanes	CH_3 (1375 cm^{-1}), (CH_2 1450 cm^{-1})
3000-3100 cm^{-1}	**C–H stretch**	Alkenes	CH_3 (1375 cm^{-1}), (CH_2 1450 cm^{-1}), (C=C 1600-1660 cm^{-1})

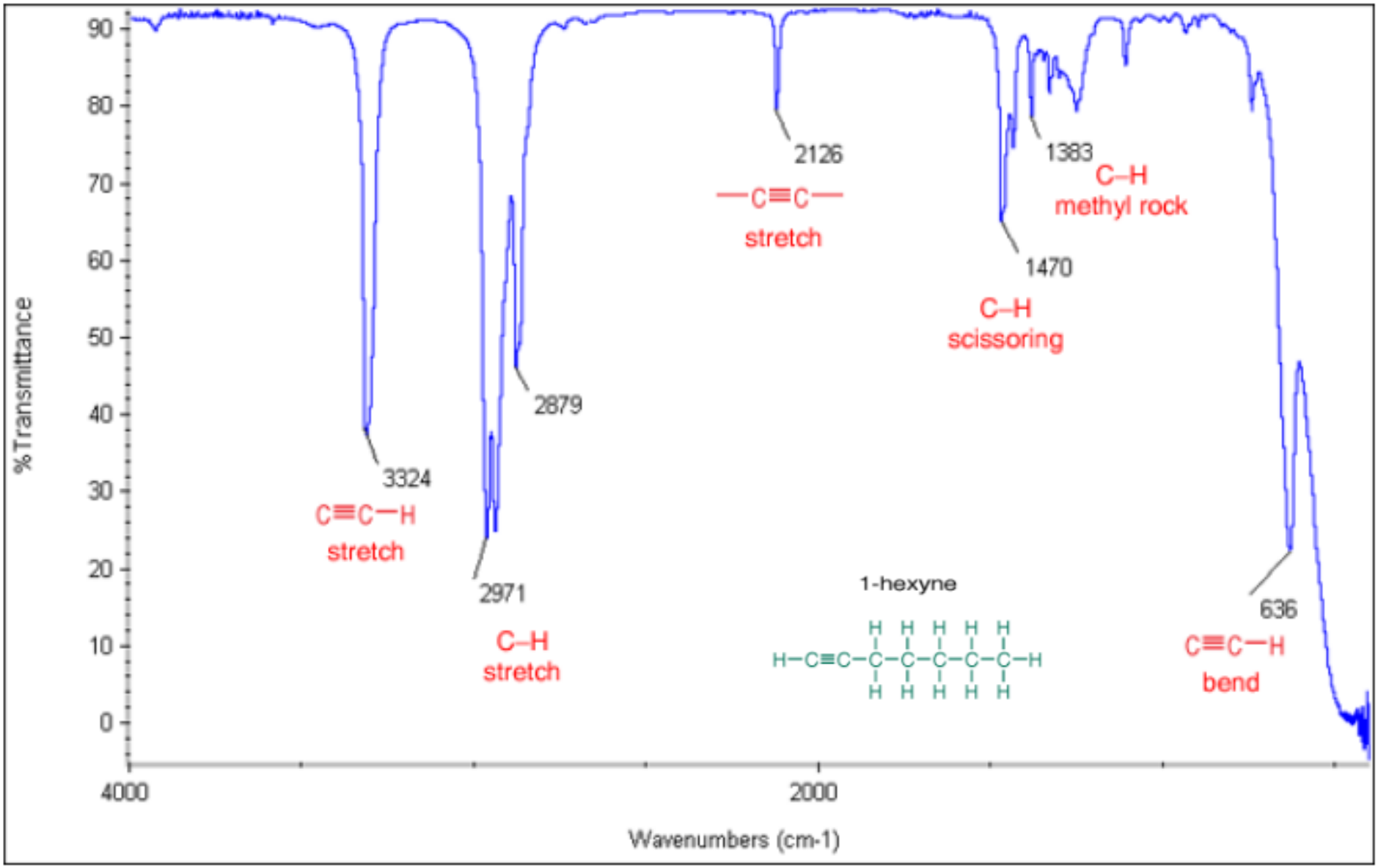
How Can We Interpret a spectrum-EX-1



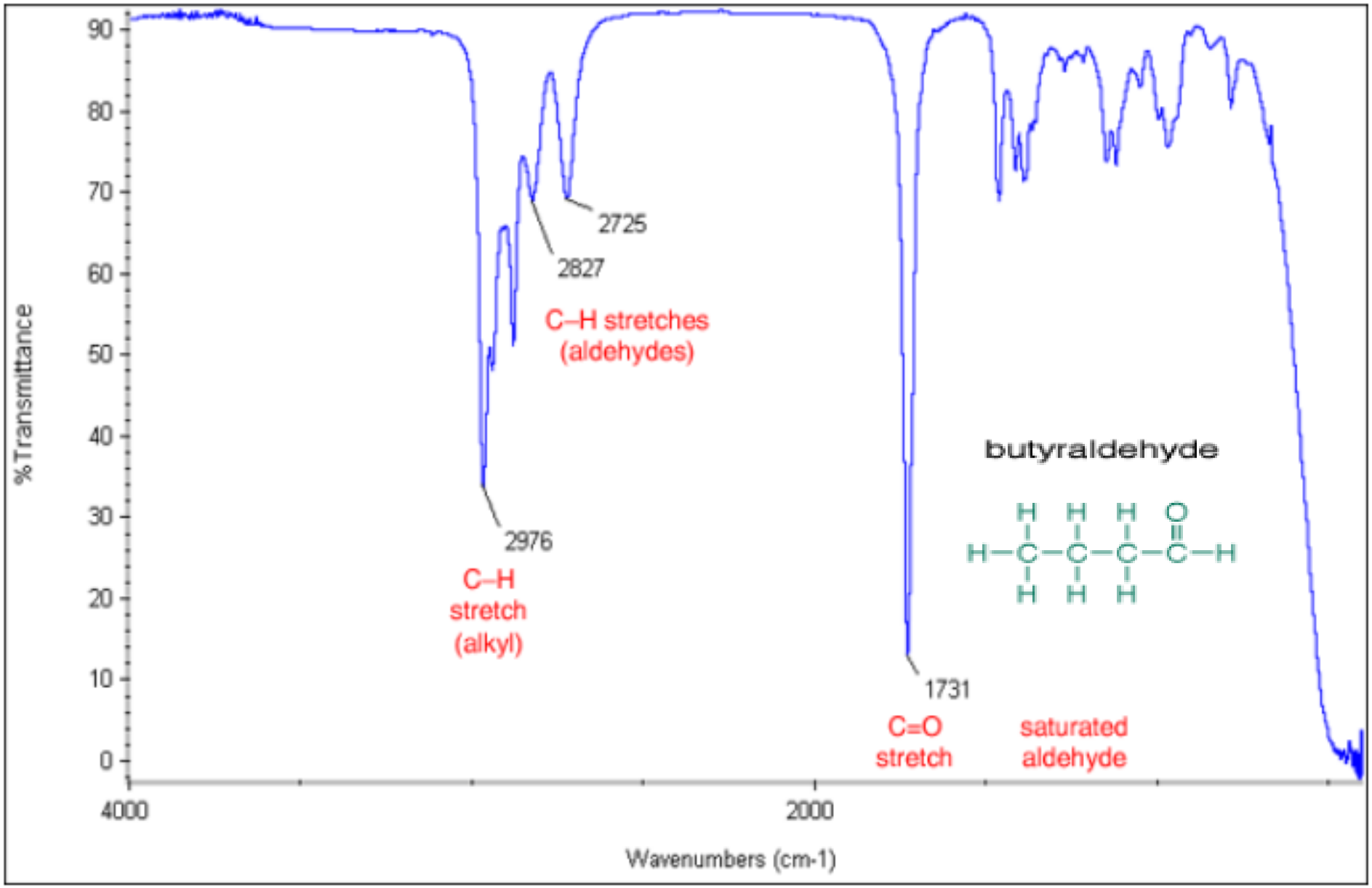
How Can We Interpret a spectrum-EX-2



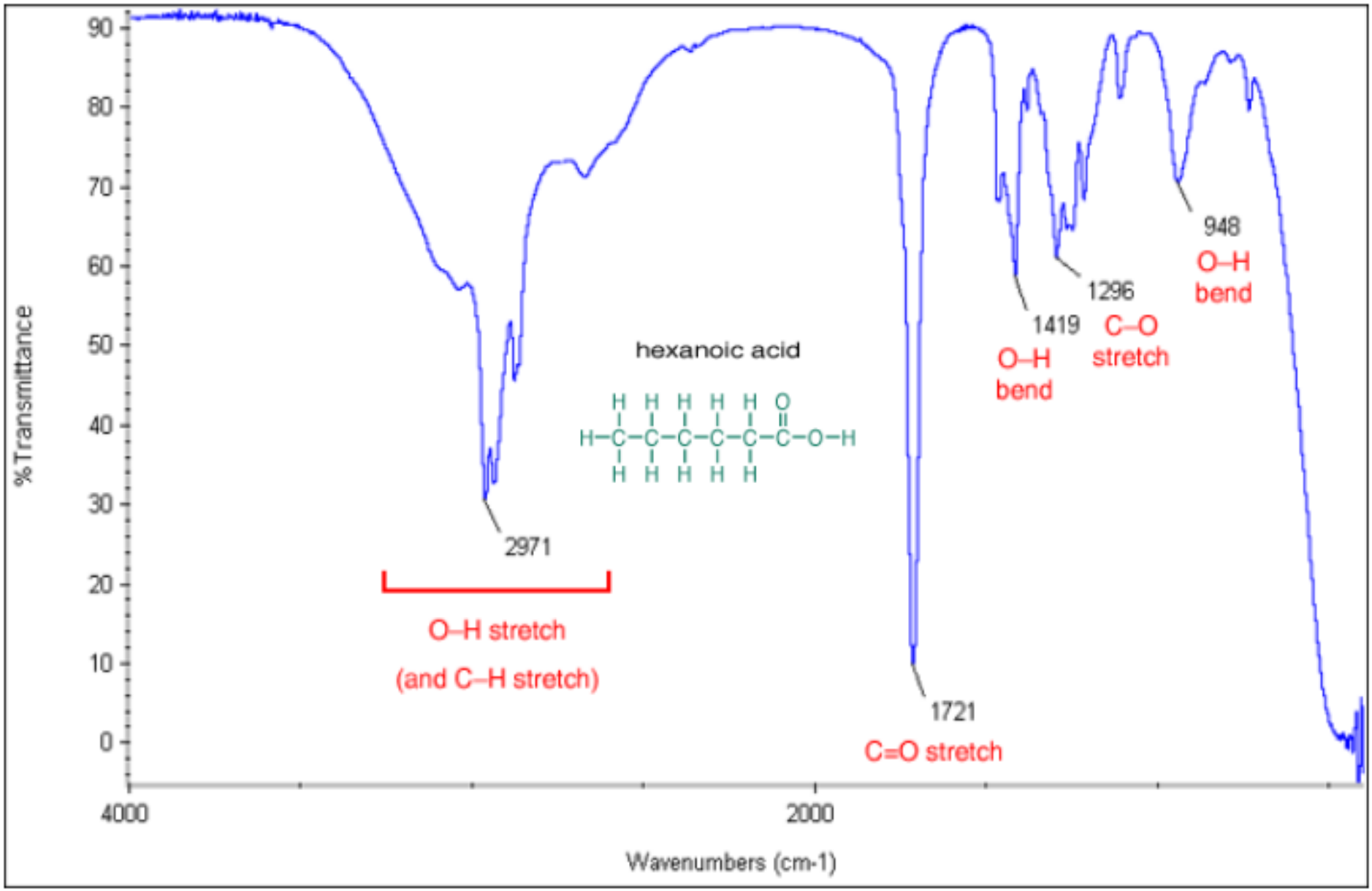
How Can We Interpret a spectrum-EX-3



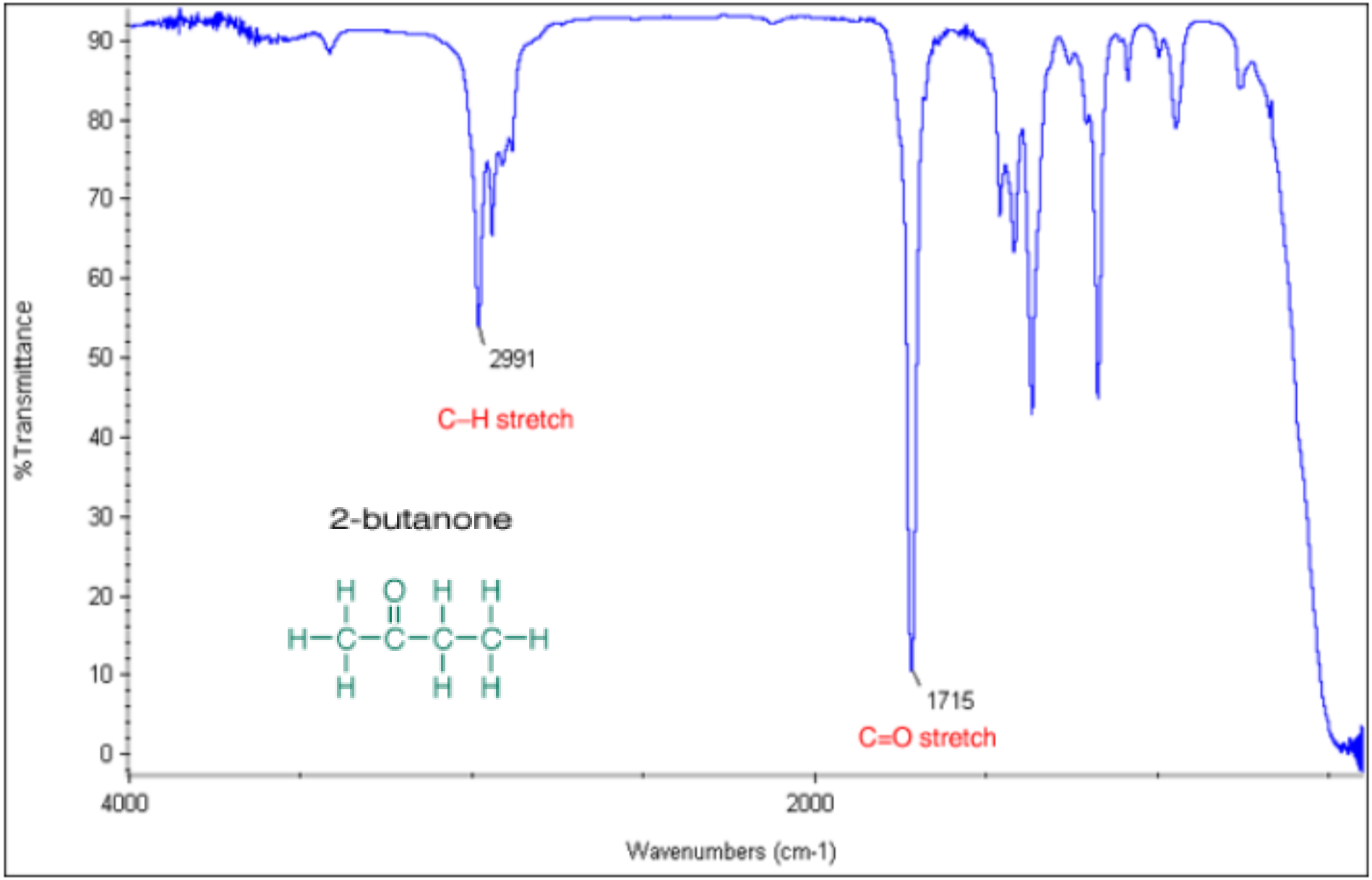
How Can We Interpret a spectrum-EX-4



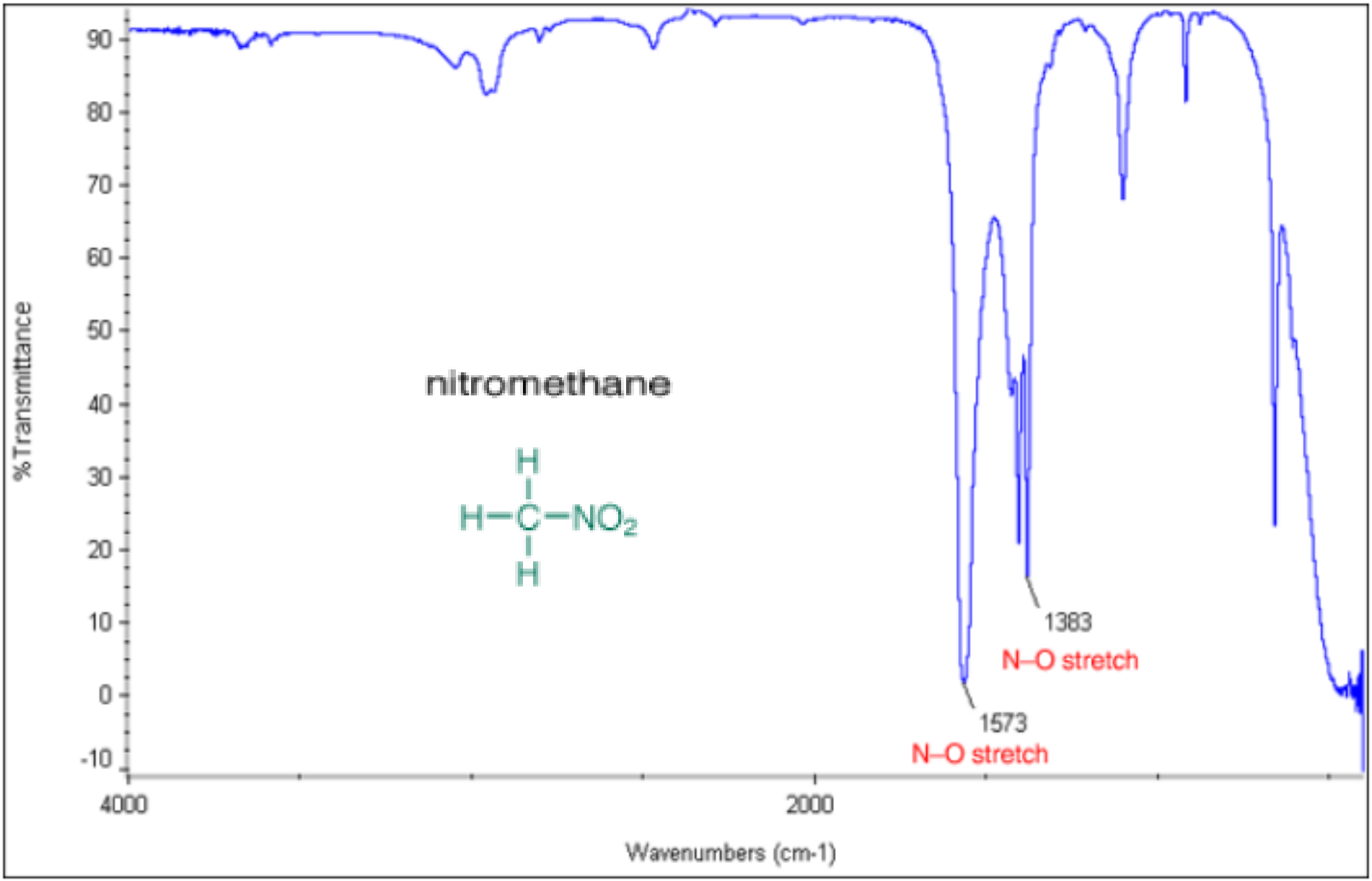
How Can We Interpret a spectrum-EX-5



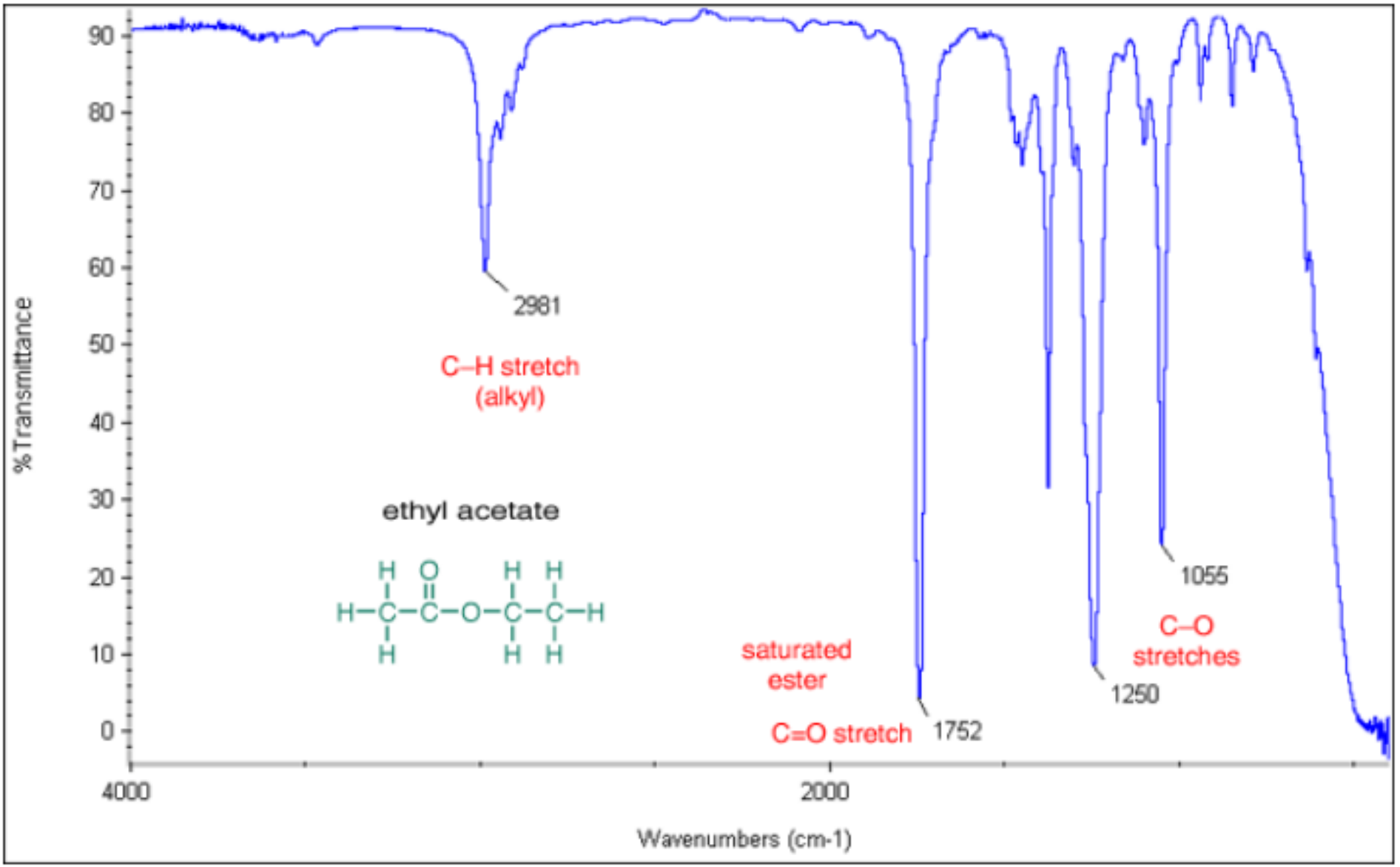
How Can We Interpret a spectrum-EX-6



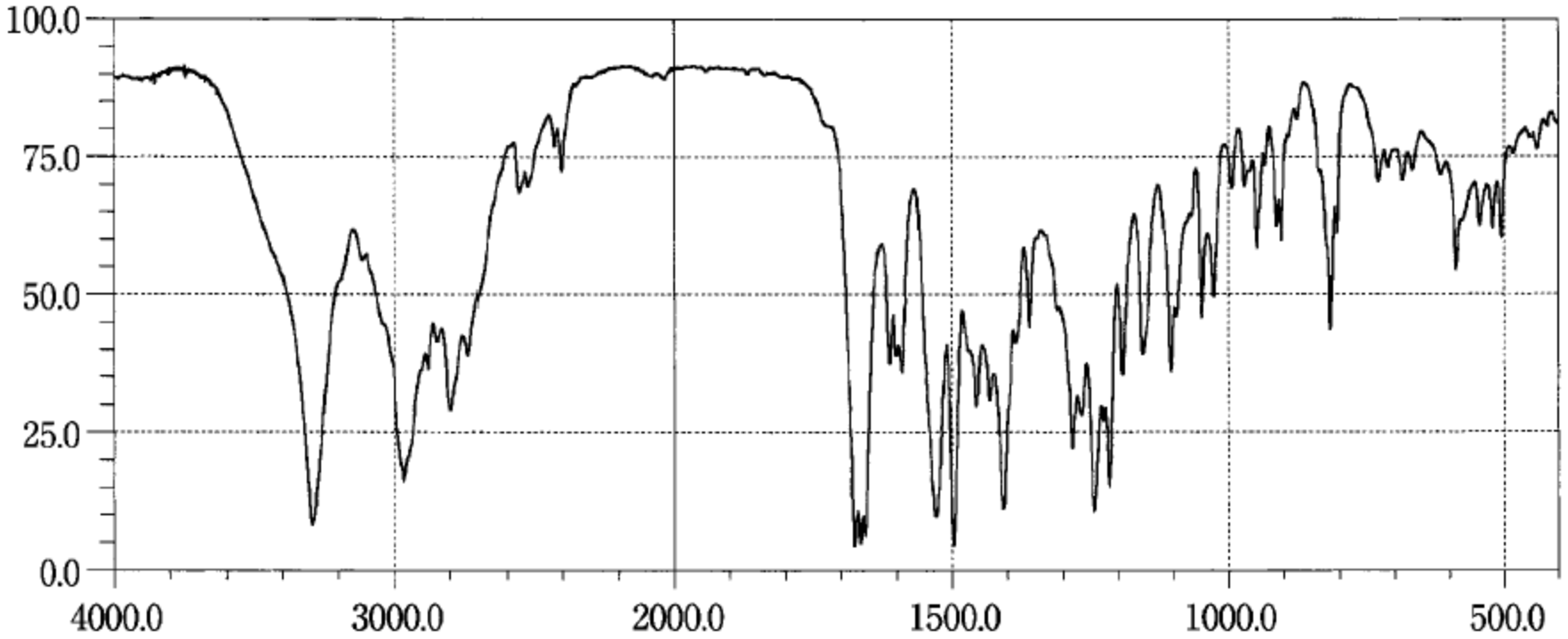
How Can We Interpret a spectrum-EX-7

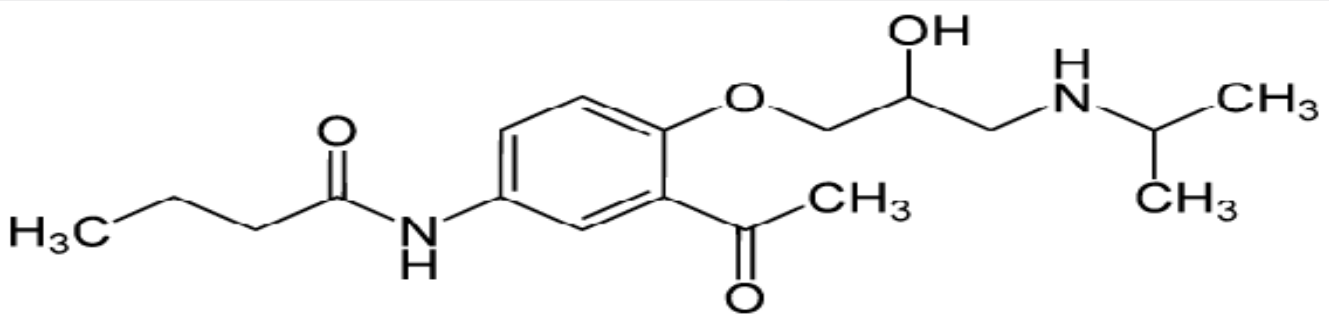


How Can We Interpret a spectrum-EX-8



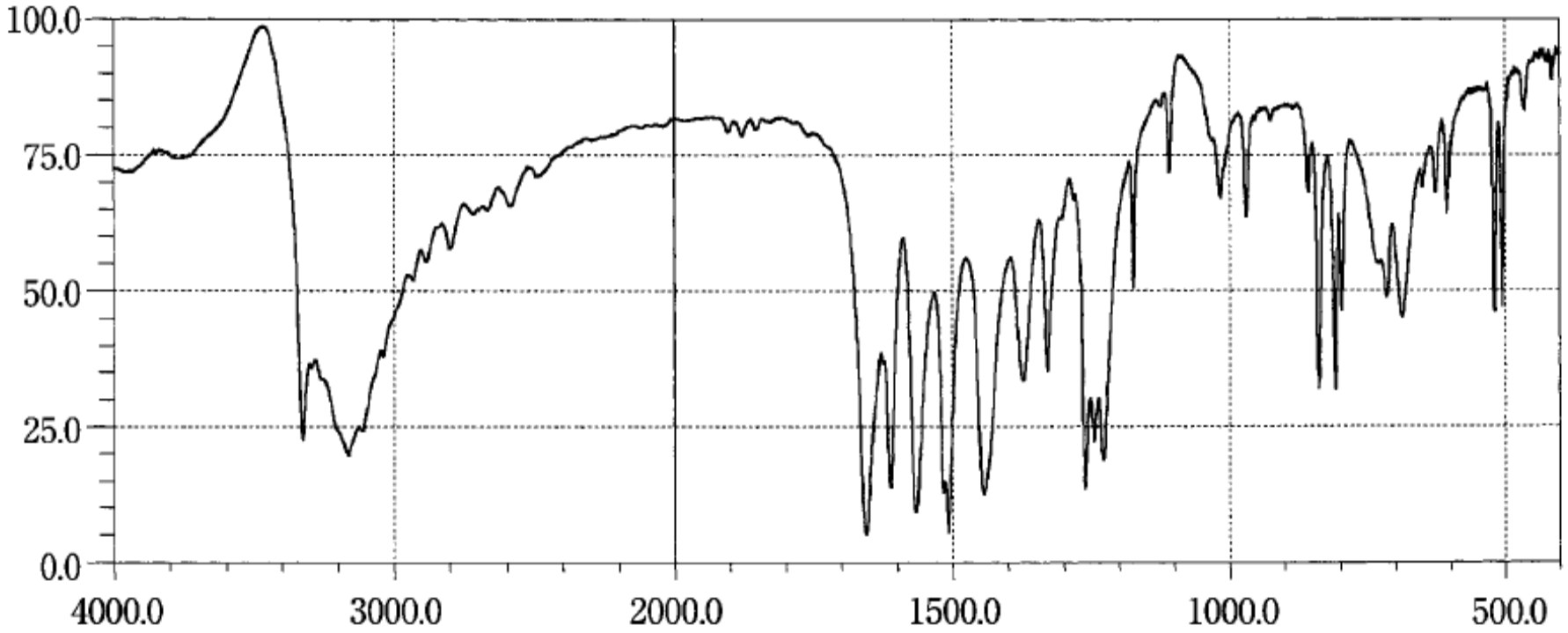
Acebutolol

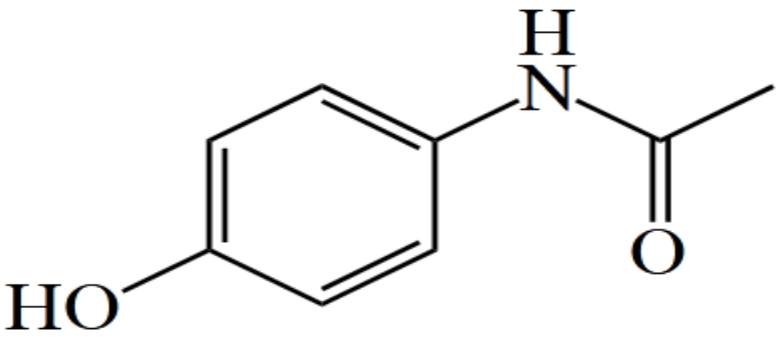




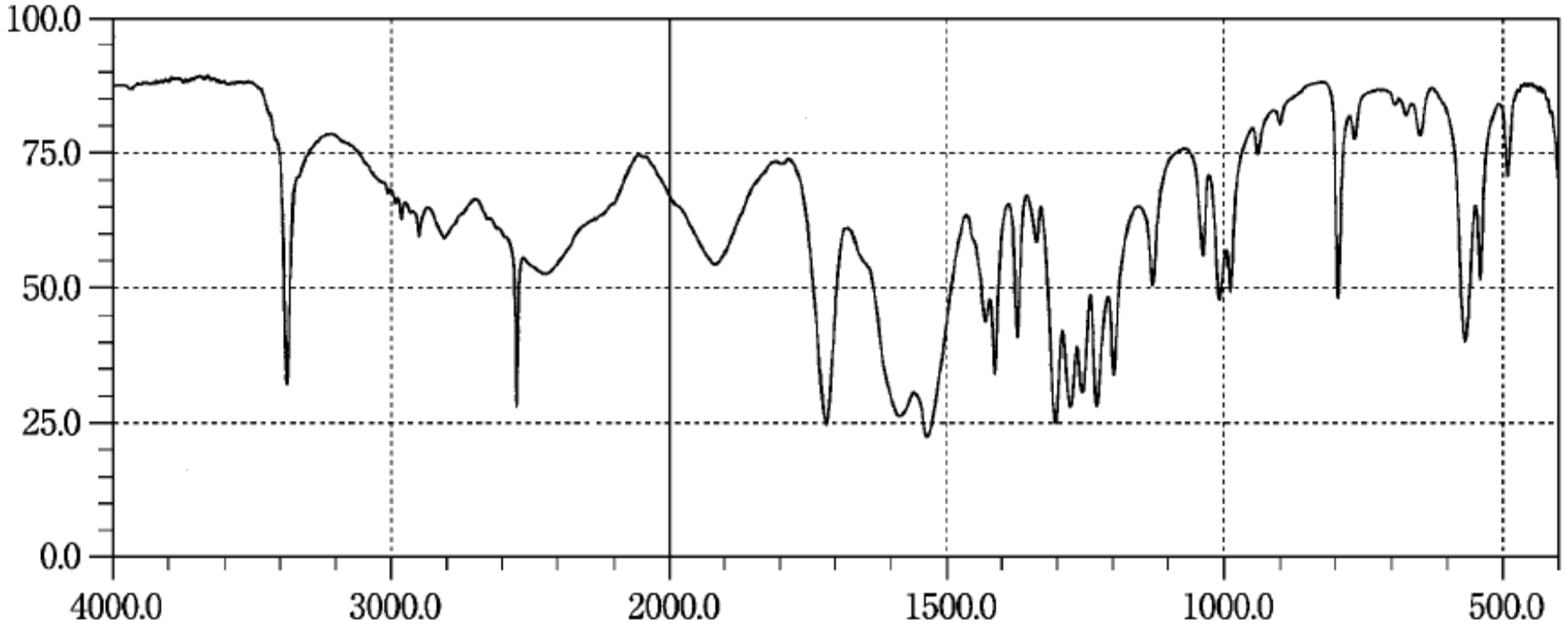
A beta blocker : treatment of hypertension and arrhythmias.

Acetaminophen

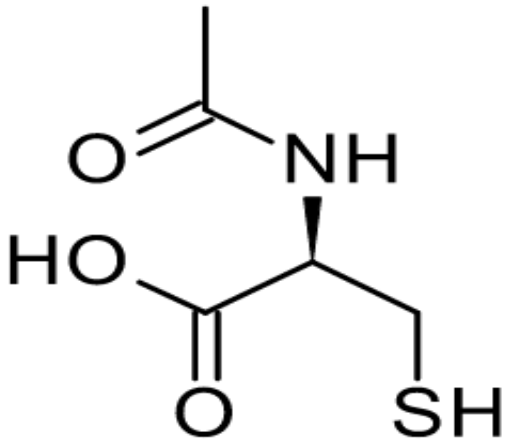




Acetylcysteine



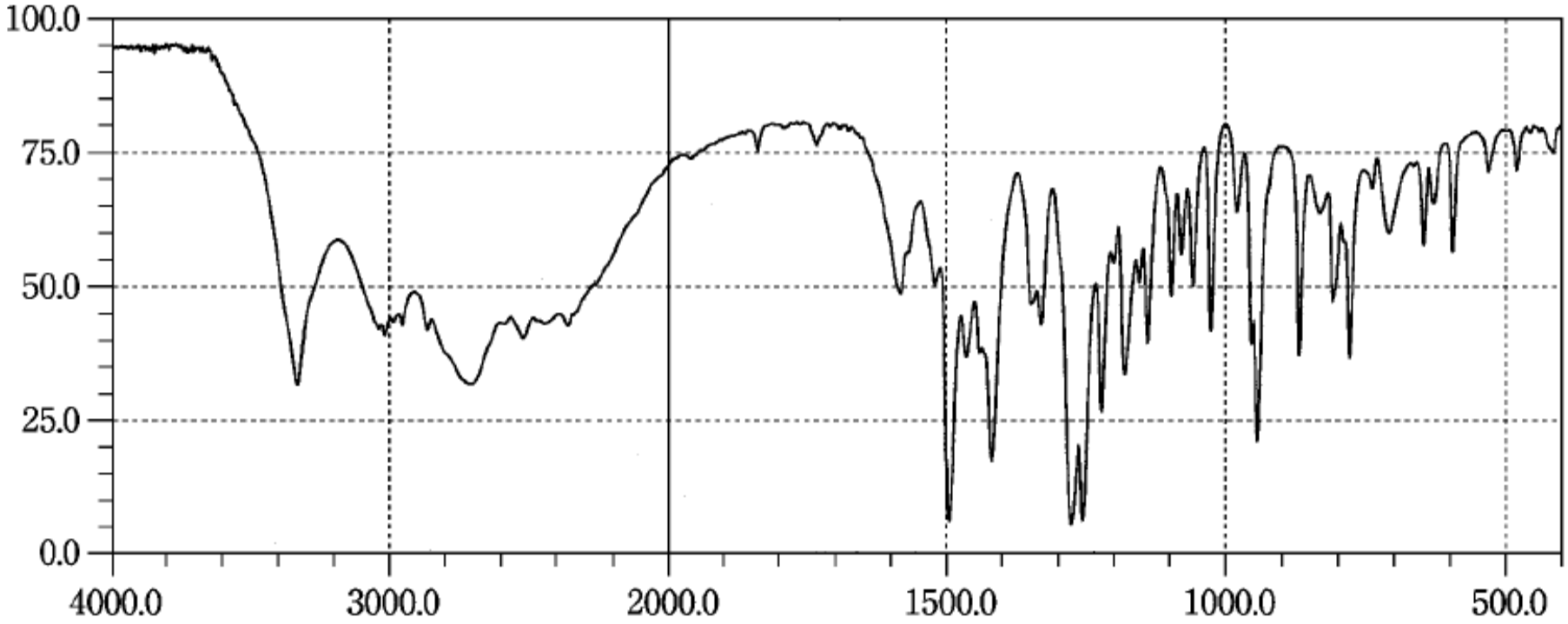
Thiol: R-SH==2550-2600 cm^{-1}

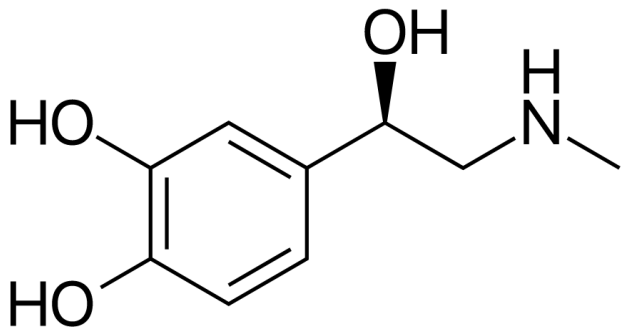


To treat Paracetamol overdose,

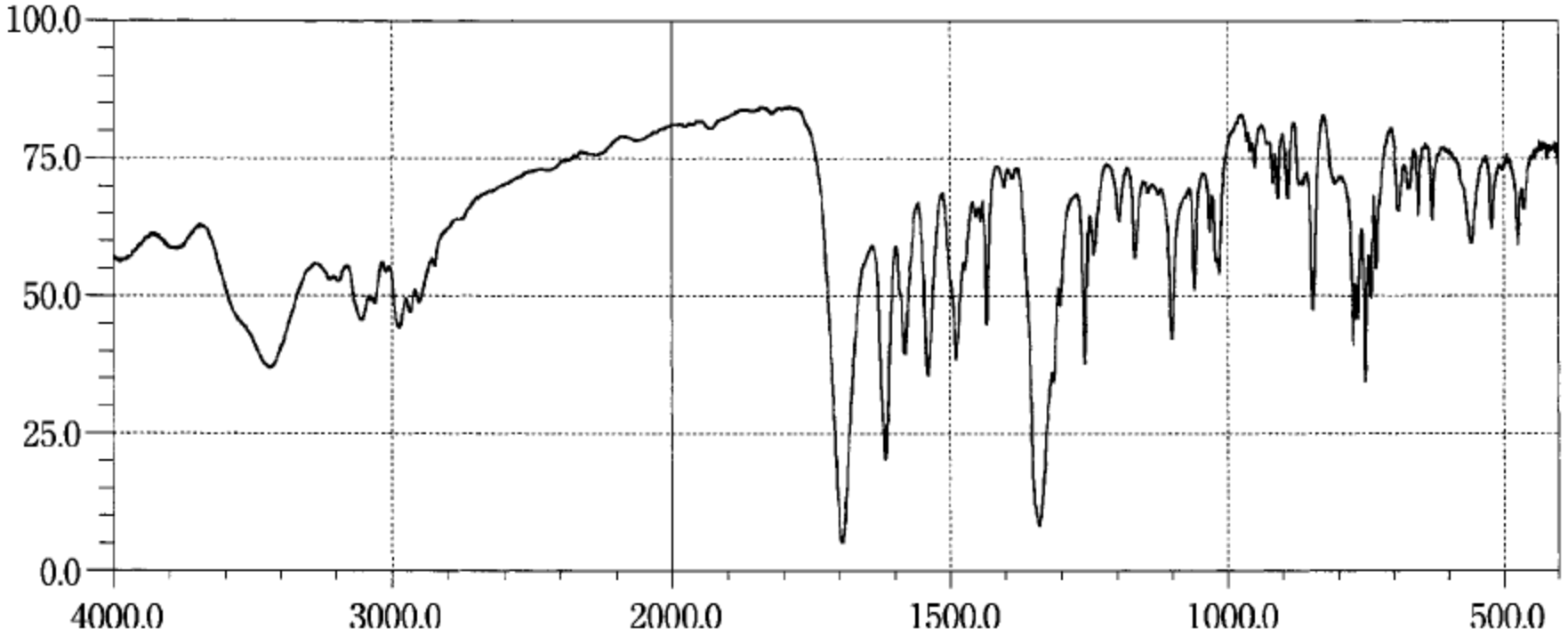
to loosen thick mucus

Adrenaline

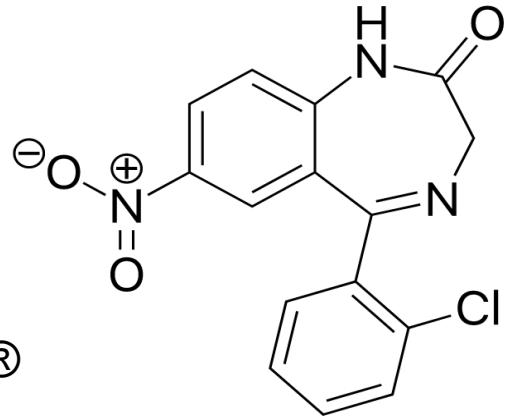

100.0
75.0
50.0
25.0
0.0
4000.0
3000.0
2000.0
1500.0
1000.0
500.0


OH
H
N
HO
HO

Clonazepam



prevent and treat seizures, panic disorder, and the movement disorder known as akathisia



Klonopin®, Ravotril®, Rivotril®, Rivatril®

Characterization Applications

Molecular absorption, particularly in the UV/Vis range, has been used for a variety of different characterization studies, including determining the stoichiometry of metal–ligand complexes and determining equilibrium constants.

❑ Stoichiometry of a Metal-Ligand Complex

The stoichiometry for a metal–ligand complexation reaction of the following general form

$$M + yL \leftrightarrow ML_y$$

❑ Determination of Equilibrium Constants

$$HIn + H_2O \leftrightarrow H_3O^+ + In^-$$

Characterization Applications

❑ Stoichiometry of a Metal-Ligand Complex

The stoichiometry for a metal–ligand complexation reaction of the following general form

$$M + yL \leftrightarrow ML_y$$

$$n(tot) = n(M) + n(L)$$

(n): number of moles,

The relative amount of ligand and metal in each solution is expressed as the mole fraction of ligand, (X_L), and the mole fraction of metal, (X_M),

$$X_L = \frac{n_L}{n_{tot}} \qquad X_M = 1 - X_L = \frac{n_M}{n_{tot}}$$

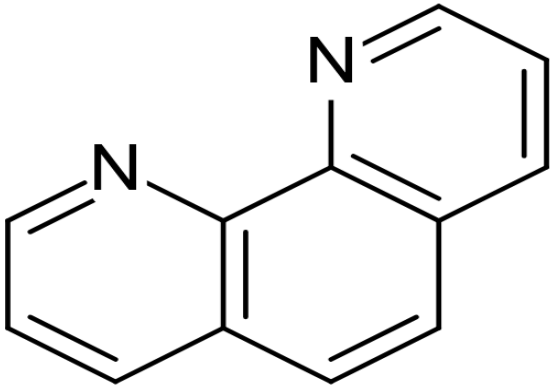
$$y = \frac{n_L}{n_M} = \frac{X_L}{X_M} = \frac{X_L}{1 - X_L}$$

Characterization Applications

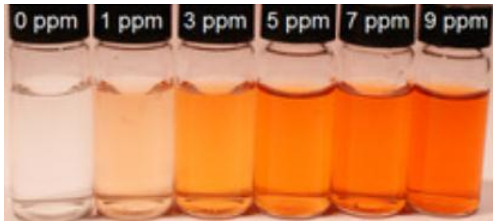
❑ Stoichiometry of a Metal-Ligand Complex: Ex.

To determine the formula for the complex between Fe^{2+} and *o*-phenanthroline, a series of solutions was prepared in which the total concentration of metal and ligand was held constant at 3.15×10^{-4} M. The absorbance of each solution was measured at a wavelength of 510 nm. Using the following data, determine the formula for the complex.

X_L	Absorbance
0	0
0.1	0.116
0.2	0.231
0.3	0.347
0.4	0.462
0.5	0.578
0.6	0.693
0.7	0.809
0.8	0.693
0.9	0.347
1	0



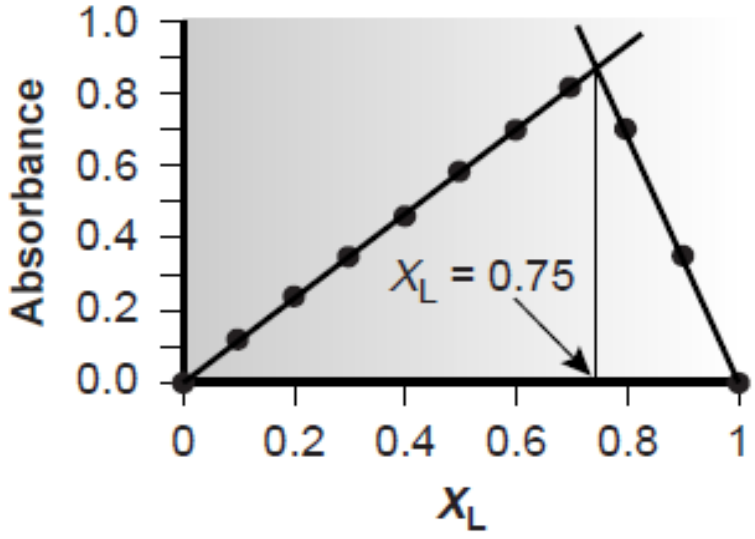
Phenanthroline



Characterization Applications

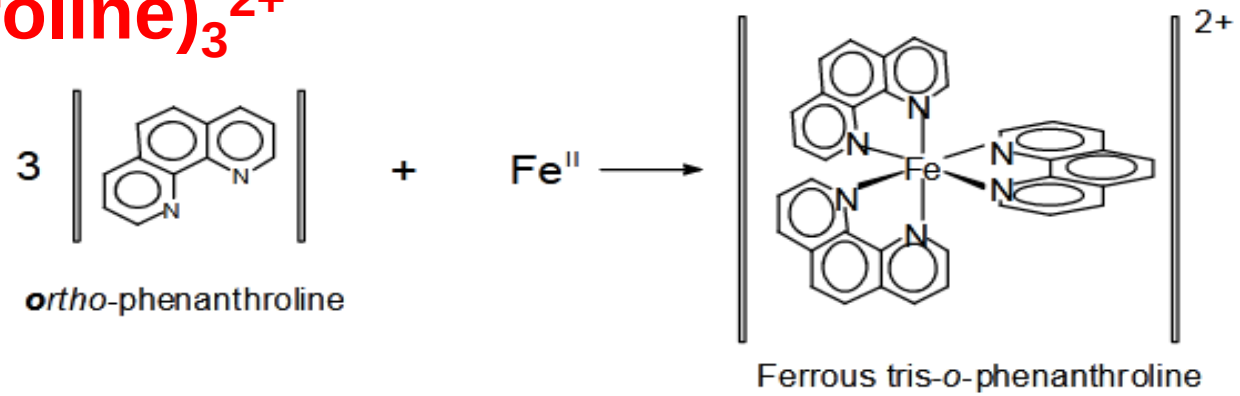
❑ Stoichiometry of a Metal-Ligand Complex: Ex.

The maximum absorbance is determined by extrapolating the two linear portions of the plotis done. X_L=0.75.



$$y = \frac{X_L}{1 - X_L} = \frac{0.75}{1 - 0.75} = 3$$

Fe(*o*-phenanthroline)$_3^{2+}$



Characterization Applications

❑ Determination of Equilibrium Constants

$$HIn + H_2O \leftrightarrow H_3O^+ + In^-$$

$$K_a = \frac{[H_3O^+][In^-]}{[HIn]}$$

$$A = \varepsilon_{HIn} b[HIn] + \varepsilon_{In} b[In^-] \qquad C = [HIn] + [In^-]$$

$$A = \varepsilon_{HIn} b(C - [In^-]) + \varepsilon_{In} b[In^-]$$

$$A = \varepsilon_{HIn} bC - \varepsilon_{HIn} b[In^-] + \varepsilon_{In} b[In^-]$$

$$A = A_{HIn} + b[In^-](\varepsilon_{In} - \varepsilon_{HIn})$$

Characterization Applications

❑ Determination of Equilibrium Constants

$$HIn + H_2O \leftrightarrow H_3O^+ + In^-$$

$$[\mathrm{In^-}] = \frac{A - A_{\mathrm{HIn}}}{b(\varepsilon_{\mathrm{In}} - \varepsilon_{\mathrm{HIn}})} \qquad [\mathrm{HIn}] = \frac{\mathrm{A_{In}} - A}{b(\varepsilon_{\mathrm{In}} - \varepsilon_{\mathrm{HIn}})}$$

$$K_a = [\mathrm{H_3O^+}]\left(\frac{A - A_{\mathrm{HIn}}}{A_{\mathrm{In}} - A}\right)$$

The simplest approach is to prepare three solutions (C constant):

1. Acidic solution: [HIn]>>[In⁻], A1=A(HIn).
2. Alkalin solution: [In⁻]>>[HIn], A2=A(In⁻)
3. Intermediate solution, A and pH are recorded.

The value of *K*a can then be calculated by making appropriate substitutions into the equation.

Characterization Applications

❑ Determination of Equilibrium Constants: EX.

The acidity constant for an acid–base indicator was determined by preparing three solutions, each of which has a total indicator concentration of 5.00×10^{-5} M.

The first solution was made strongly acidic with HCl and has an absorbance of 0.250.

The second solution was made strongly basic and has an absorbance of 1.40.

The pH of the third solution was measured at 2.91, with an absorbance of 0.662. What is the value of K_a for the indicator?

$$k_a = (1.23 \times 10^{-3})\left(\frac{0.662 - 0.250}{1.40 - 0.662}\right) = 6.87 \times 10^{-4}$$

Quantitative Measurements From IR Spectra

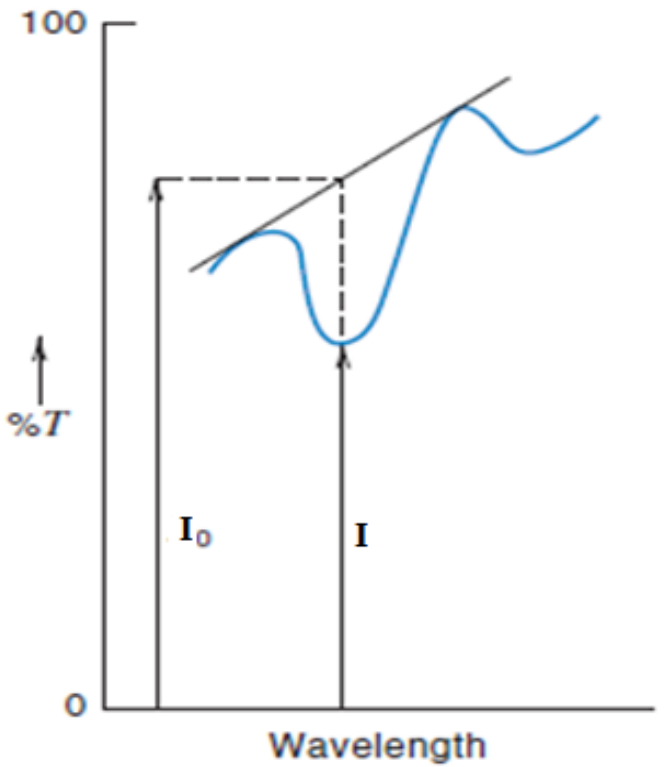
IR spectrometry is normally used to identify functional groups. However, QUANTIFICATION can also be performed

Difficult to use the Beer's law: scattered radiation, weak sources (wide slits is needed)

The **baseline** or **ratio method**

Log I_0/I is plotted against concentration in the usual manner.

Unknowns are compared against standards run under the same instrumental conditions.



Luminescence

Luminescence is a spontaneous emission of light by a substance not resulting from heat. (Cold light)

Types of luminescence:

- **Photoluminescence**: emission of light following excitation by photons, (Fluorescence and phosphorescence),
- **Radioluminescence**: excitation by energetic radiation (Gamma rays, etc.).
- **Electroluminescence:** electrical excitation,
- **Piezoluminescence**: luminescence created by pressure,

Luminescence

Types of luminescence:

- **Thermoluminescence:** (misnomer); it does not really involve excitation by heat. Rather, exposure to omnipresent energetic radiation (cosmic rays) creates lattice defects in solids that are released in the form of luminescence when the material is heated.
- **Triboluminescence:** generated when material is pulled apart, ripped, scratched, crushed, or rubbed from energy released due to the breaking of chemical bonds.
- **Chemiluminescence** provides electronic excitation by energy derived from a chemical reaction; such reactions may be brought about in natural biological systems (**bioluminescence**, as in fireflies).

Luminescence

bioluminescence

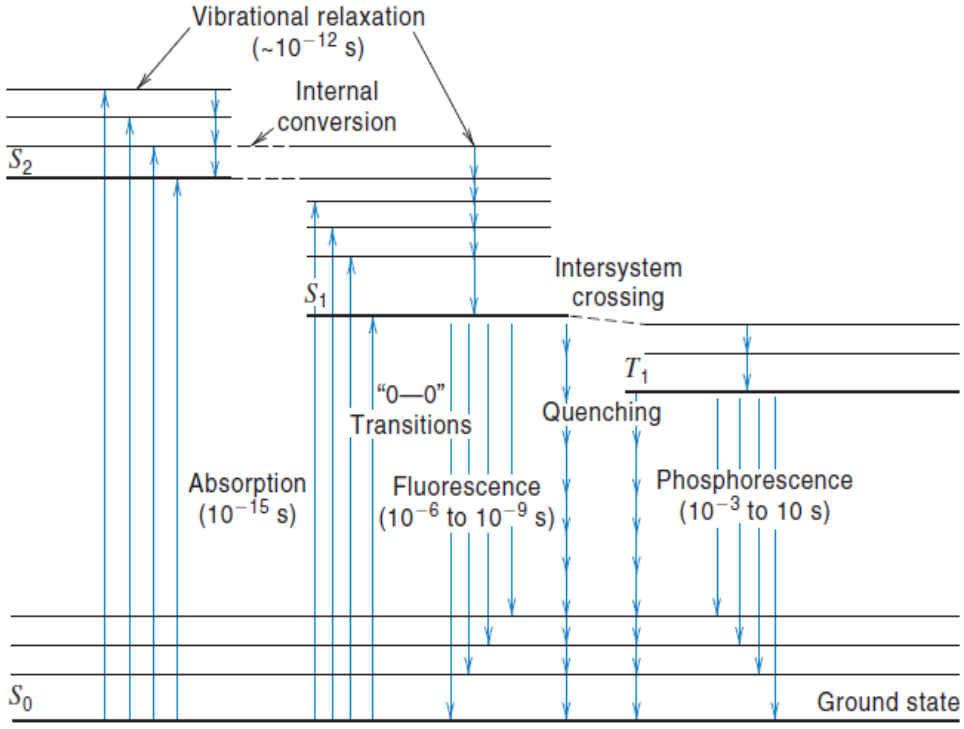
Luminescence

Fluorescence

Fluorometry

Some molecules that absorb UV radiation lose only part of the absorbed energy by collisions. The rest energy is reemitted as radiation at longer wavelengths.

5 to 10% of all molecules fluoresce, especially when excited by energetic UV radiation.



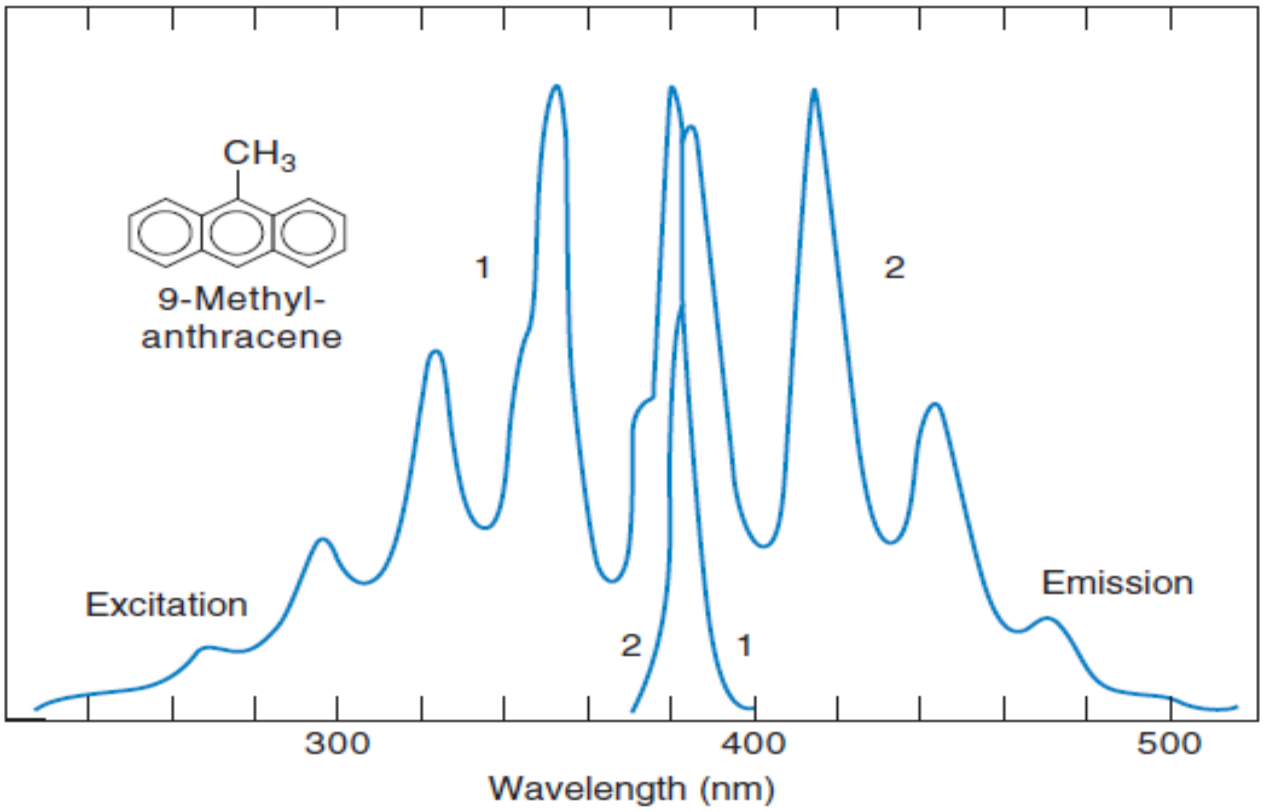
1. Internal conversion,
2. Vibrational relaxation
3. Fluorescence

Fluorescence: (10^{-6} to 10^{-9} s). the electron drops back to the ground state by emitting a photon of lower energy (longer wavelength) than was absorbed.

Fluorometry

Fluorescence: A typical excitation and emission spectrum of a fluorescing molecule.

9-Methylanthracene



Stokes shift

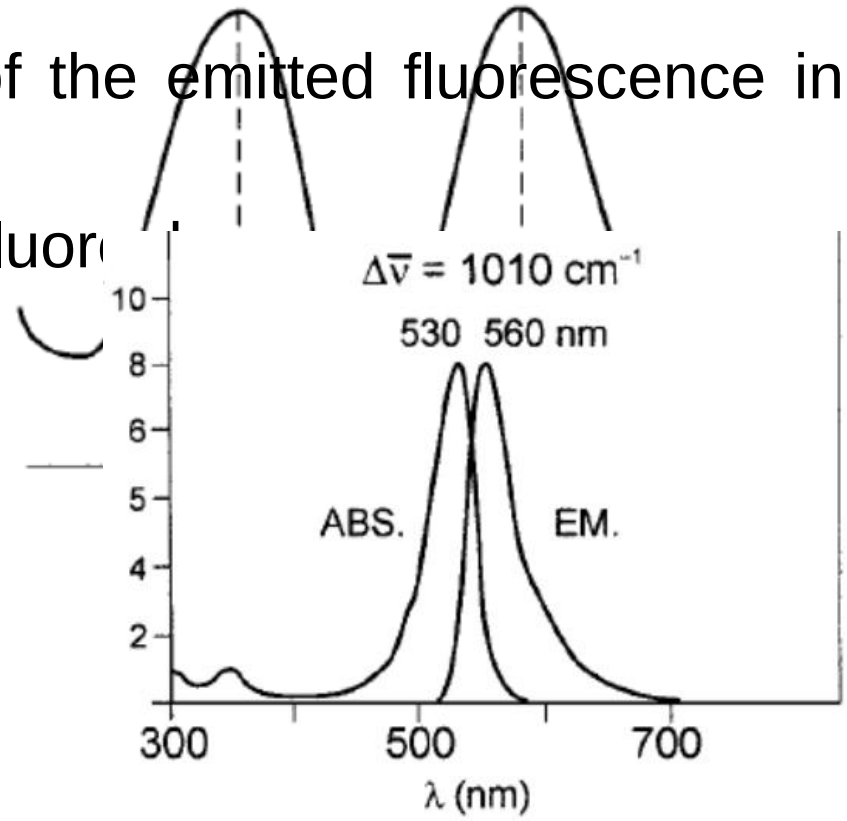
The Stokes shift ($\Delta\tilde{\nu}$): the distance (in wavenumbers) between the maximum of the first absorption band and the maximum of fluorescence.

The Stokes Shift is:

- ✓ A key aspect in the detection of the emitted fluorescence in biological applications.
- ✓ A distinct characteristic of each fluor

Example

Calculate the stockes shift .



Fluorometry

Chemical structure and fluorescence

✓Any molecule that absorbs radiation and is promoted to an electronically excited state **could** fluoresce.

✓The greater the absorption by a molecule, the greater its fluorescence intensity.

✓Many aromatic and heterocyclic compounds fluoresce, particularly if they contain certain substituted groups.

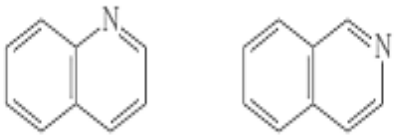
conjugated double bonds enhance fluorescence:

❑ One or more electron-donating groups such as —OH, —NH_2, and —OCH_3.

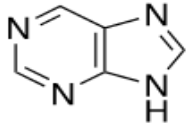
Chemical structure and fluorescence

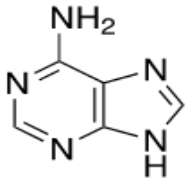
- Polycyclic compounds such as vitamin K, purines, and nucleosides and conjugated polyenes such as vitamin A are fluorescent.

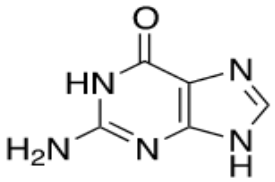
Purines

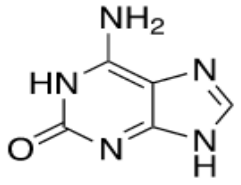

quinoline

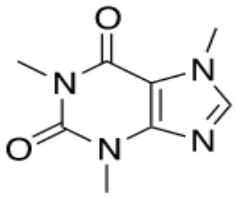
isoquinoline

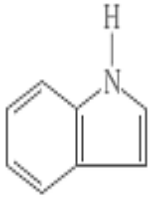

purine

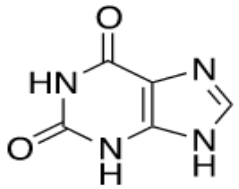

adenine

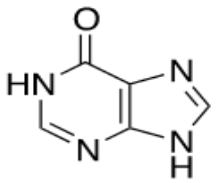

guanine

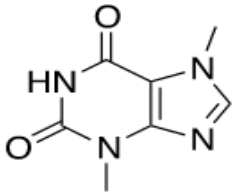

isoguanine

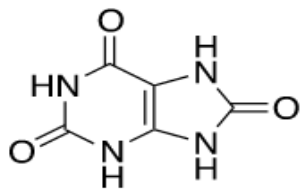

caffeine


indole

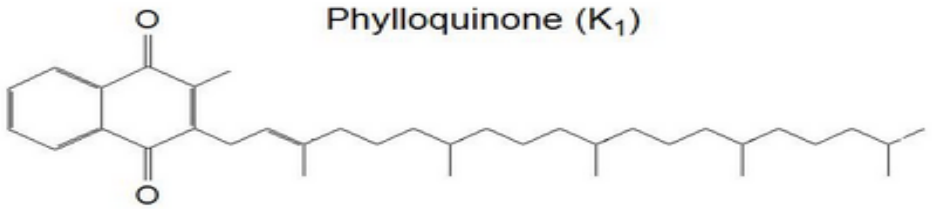

xanthine


hypoxanthine


theobromine


uric acid

Vitamin K

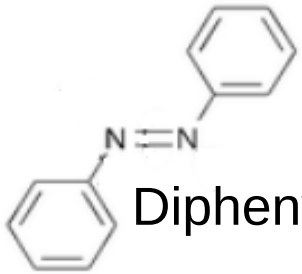


Chemical structure and fluorescence

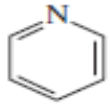
❑ Groups inhibit fluorescence:

-NO_2, -COOH, -I,

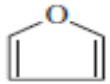
-Br, -CH_2COOH, azo groups.



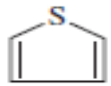
Diphenyldiaze



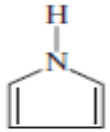
pyridine



furan



thiophene



pyrrole

❑ If a compound is nonfluorescent, it may be converted to a fluorescent derivative. Example: nonfluorescent steroids may be converted to fluorescent compounds by dehydration with concentrated H_2SO_4.

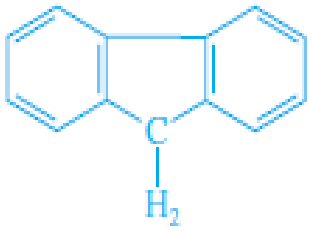
❑ Fluorescence of many molecules is greatly pH dependent, because only the ionized or un-ionized form may be fluorescent.

Example: phenol, (C_6H_5OH) is fluorescent, but its anion ($C_6H_5O^-$), is not.

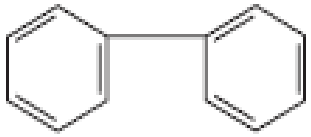
Chemical structure and fluorescence

Effect of molecular rigidity on quantum yield

Fluorine molecule is held rigid by the central ring, fluorescence is enhanced. The planes of the two rings in biphenyl rotate relative to one another, fluorescence is suppressed.

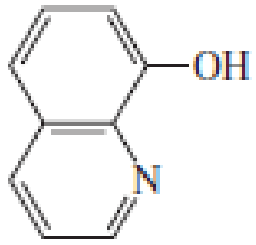


fluorene
$\Phi \rightarrow 1$

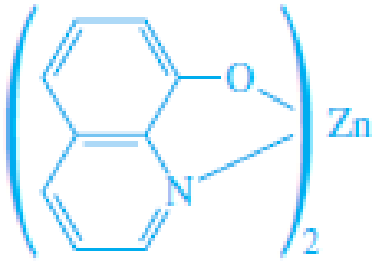


biphenyl
$\Phi \rightarrow 0.2$

More rigid = More fluorescence



nonfluorescing



fluorescing

❑ **Effect of rigidity on quantum yield in complexes**.

Free 8-hydroxyquinoline molecules in solution are easily deactivated through collisions with solvent molecules and do not fluoresce. The rigidity of the Zn-8-hydroxyquinoline complex enhances fluorescence.

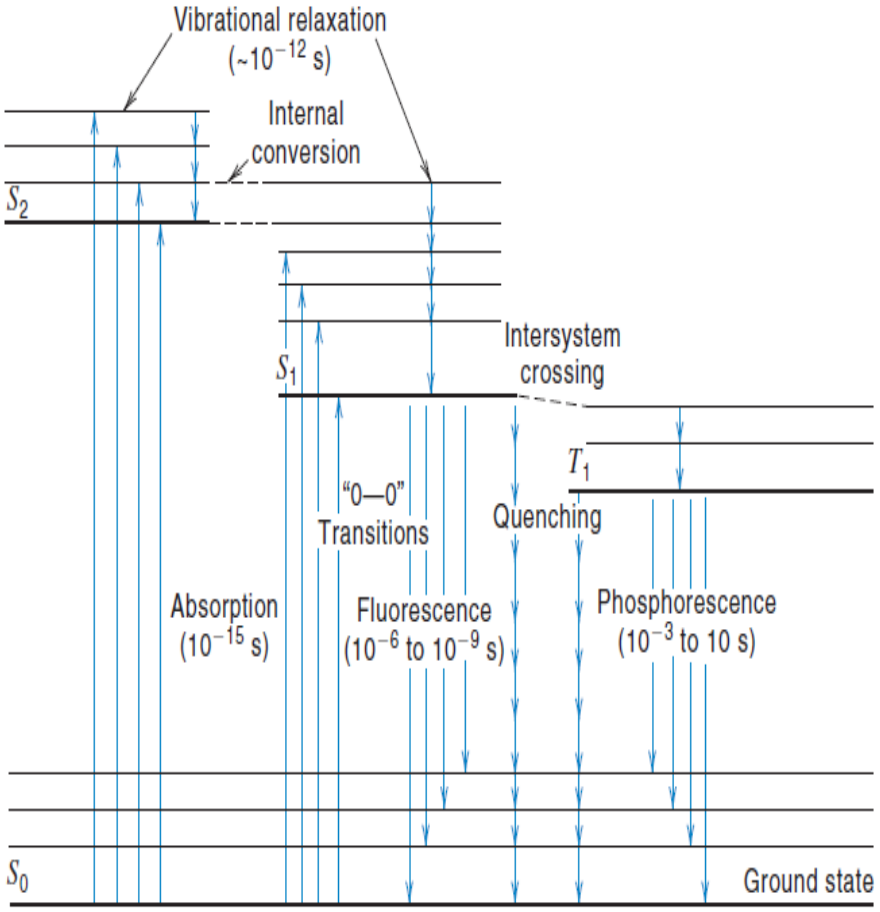
Chemical structure and fluorescence

❑ Fluorescence Quenching

Quenching of fluorescence is often a problem in quantitative measurements.

❑Substances that compete for the electronic excitation energy and decrease the quantum yield (Ø: the efficiency of conversion of absorbed radiation to fluorescent radiation).

Iodine and bromine substituents decrease the quantum yield (extremely effective quencher).



Chemical structure and fluorescence

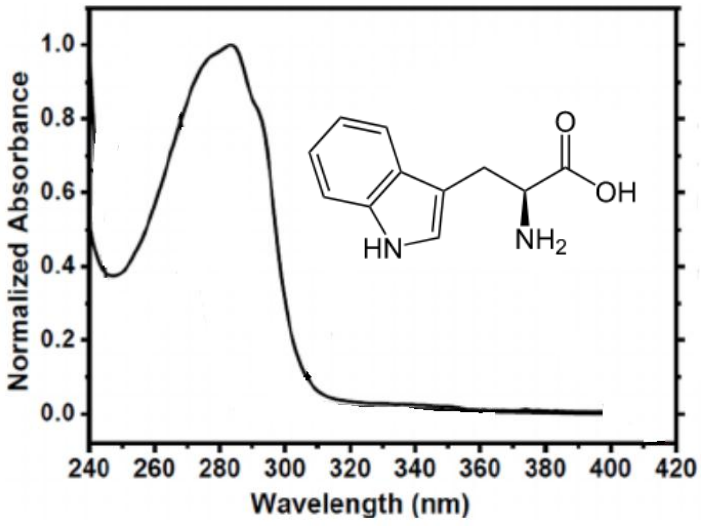
❑ Inner-filter effect

A colored species in solution with a fluorescing analyte may interfere by absorbing the excitation radiation or the emitted fluorescent radiation or both.

Example, in Na_2CO_3 solution, the ($K_2Cr_2O_7$) exhibits absorption peaks at 245 and 348 nm. These overlap with the excitation and emission peaks for tryptophan.

✓ The inner-filter effect can arise from too high concentration of the fluorophore itself. Some of the analyte molecules will reabsorb the emitted radiation of others (non linear calibration curve ;(.

Tryptophan



Fluorescence Measurement

$$F = \phi I_0 (1 - 10^{-\varepsilon b c})$$

F ≡ the fluorescence intensity,

Ø ≡ the quantum yield,

- The quantum yield is high, the majority of light absorbed is found in fluorescent emission,
- The quantum yield is small, most absorbed energy is lost by thermal effect.

Remember

ε ≡ Molar absorptivity (or extinction coefficient)

b ≡ path length light travels through cuvet

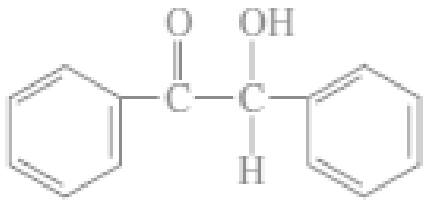
c ≡ concentration of analyte

Fluorescence Application

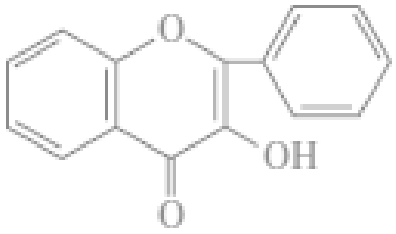
- Analysis of amino acids, proteins, coenzymes, vitamins, nucleic acids, alkaloids, etc.
- Metal cations analysis: Al, Be (8-hydroxyquinoline),

Zr, Sn (Favanol),

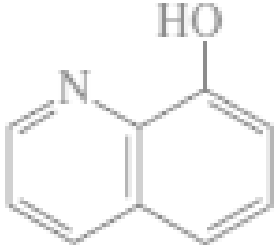
B, Zn, Ge, Si (Benzoin).



Benzoin



Favanol



8-hydroxyquinoline

Fluorescence vs. Absorbance

Fluorometric methods

Absorption spectrometry

Sensitivity

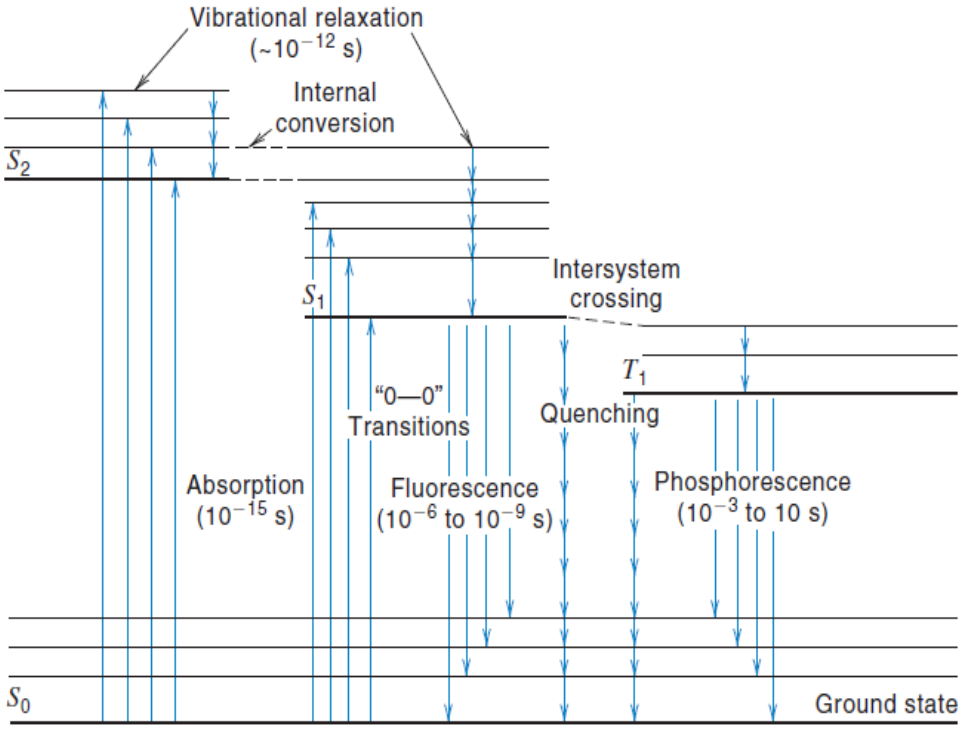
It is possible to detect a single molecule, a feat not matched by any other technique.

Phosphorescence

Phosphorescence: (10^{-3} to 10 s).

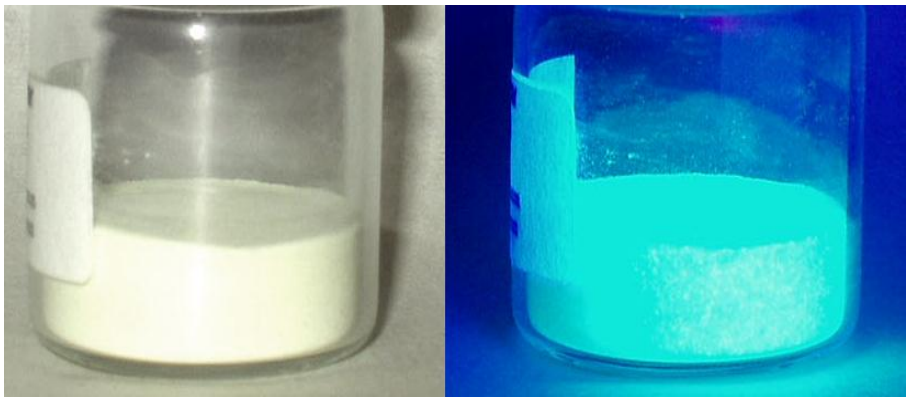
phosphorence is much longer lived than fluorescence, Consequently, “afterglow” in phosphorescence can be perceived when the excitation source is removed.

1. Internal conversion,
2. Vibrational relaxation
3. Intersystem crossing
4. Phosphorescence



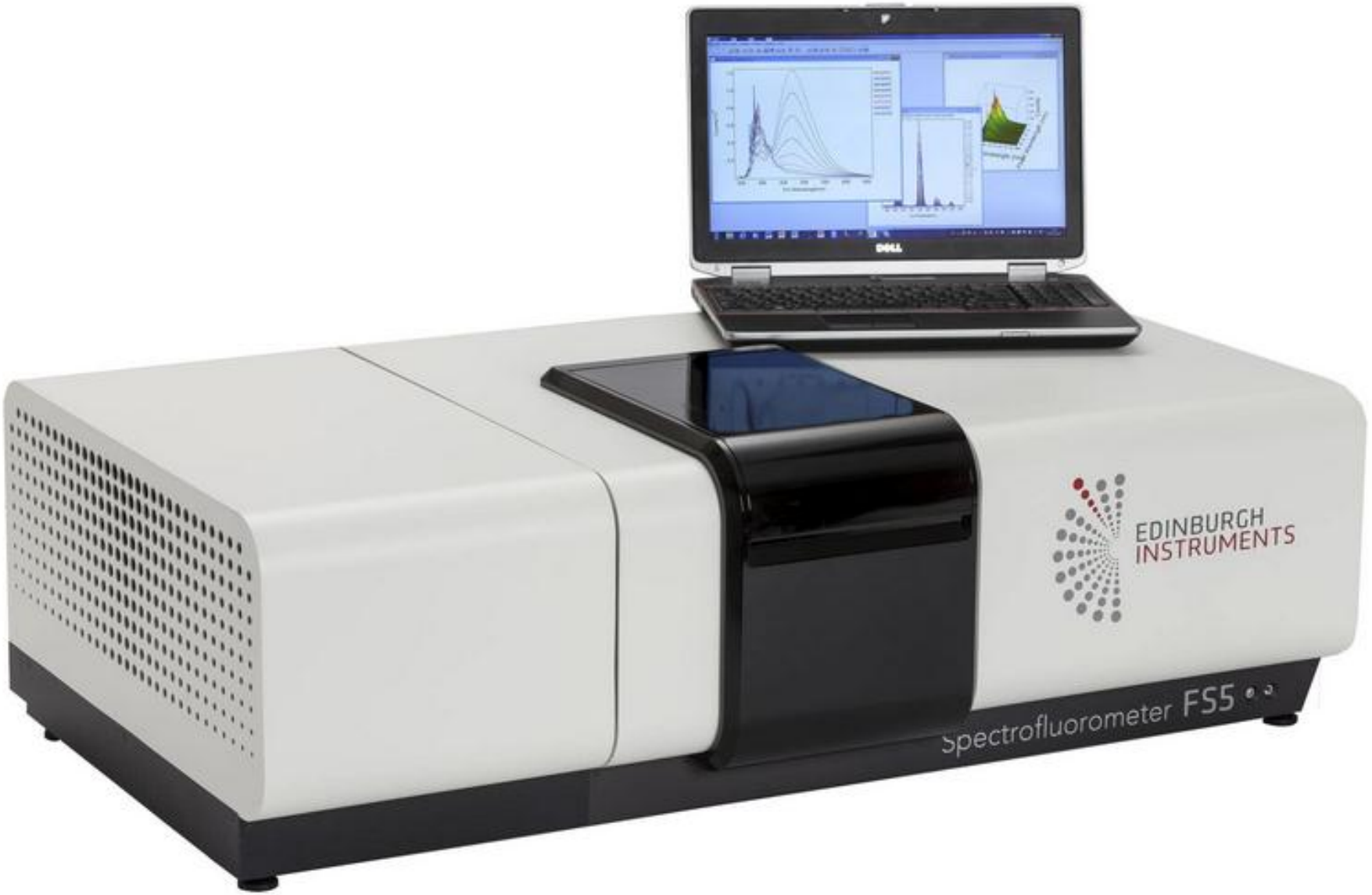
Phosphorescence

- **Zinc sulfide (ZnS)**
- **Strontium aluminate ($SrAl_2O_4$)**



Strontium aluminate ($SrAl_2O_4$)

Fluorescence and Phosphorescence Meas.



Fluorescence and Phosphorescence Meas.

- Must have intense source (D_2 or W not intense enough): use Xe or Hg_2,
- Sensitive detectors are needed (PMT): Fluorescence is not as strong as absorption,
- Phosphorescence measurements may be made on fluorimeters with 2 differences:

1. Irradiation + time delay + measurement,
2. Cooled samples (77K) to prevent collisional deactivation.

Source	Wavelength Region	Useful for
H_2 and D_2 lamp	continuum source from 160–380 nm	UV molecular absorption
tungsten lamp	continuum source from 320–2400 nm	Vis molecular absorption
Xe arc lamp	continuum source from 200–1000 nm	molecular fluorescence
Nernst glower	continuum source from 0.4–20 μm	IR molecular absorption
globar	continuum source from 1–40 μm	IR molecular absorption
nichrome wire	continuum source from 0.75–20 μm	IR molecular absorption
hollow cathode lamp	line source in UV/Vis	atomic absorption
Hg vapor lamp	line source in UV/Vis	molecular fluorescence
laser	line source in UV/Vis	atomic and molecular absorption, fluorescence and scattering

Fluorescence and Phosphorescence Meas.

Methods of determination

- Direct methods: natural fluorescence of the fluorecent sample is measured,
- Indirect methods: the nonfluorescent compound is converted into a fluorescent derivative by specific reaction or marked with fluorescent dye by attaching dye to the studied substance,
- Quenching methods: analytical signal is the reduction in the intensity of some fluorescent dye due to the quenching action of the measured sample.

Fluorescence and Phosphorescence Meas.

Applications

- Protein conformation,
- Membrane potential,
- Membrane transport,
- Membrane viscosity,
- Enzymatic reactions,
- DNA analysis,
- Genetic engineering (manipulations),
- Immunochemical methods,
- Cell proliferation and apoptosis.

Fluorescent microscopes

Suitable for fluorescence microscopy: fluorescent sample,

Several methods for creating a fluorescent sample,

The main techniques are labeling with fluorescent stains,

Biological samples, expression of a fluorescent protein.

Fluorescent protein

1. Green fluorescent protein (GFP)
2. Yellow fluorescent protein (YFP)
3. Red fluorescent protein (RFP)

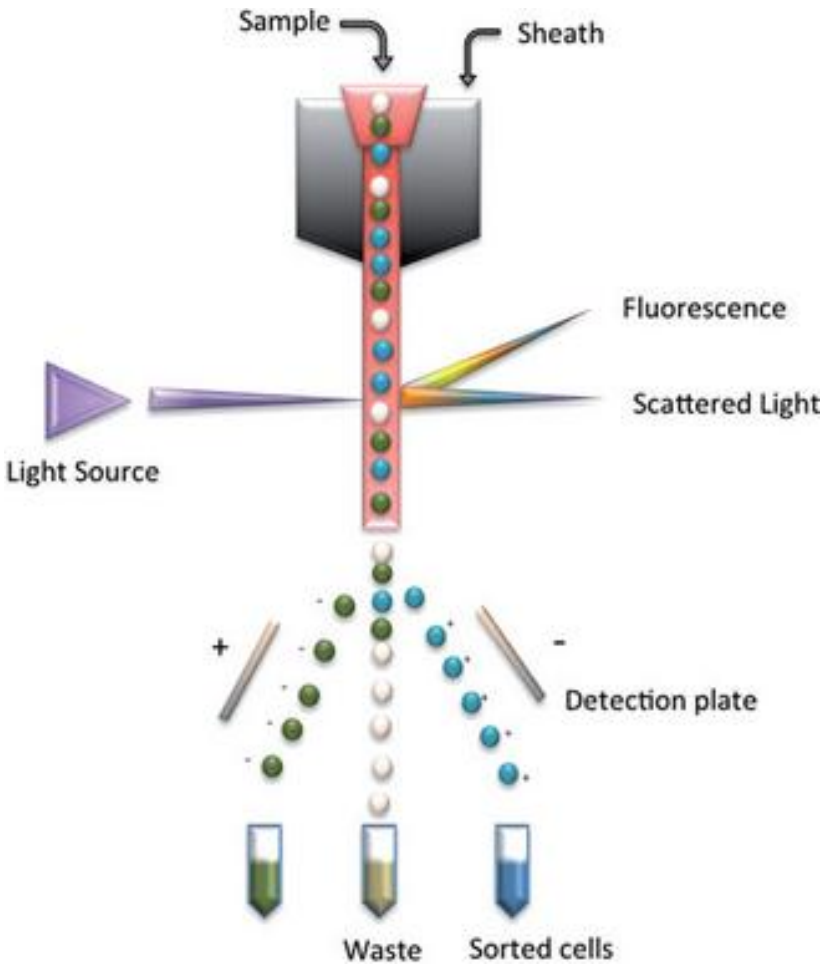
Flow cytometry

A technique used to detect and measure physical and chemical characteristics of a population of cells or particles.

- ✓ Cell counting
- ✓ Cell sorting
- ✓ Cell characteristics and function
- ✓ Detecting microorganisms
- ✓ Biomarker detection
- ✓ Protein engineering detection
- ✓ Diagnosis of health disorders:

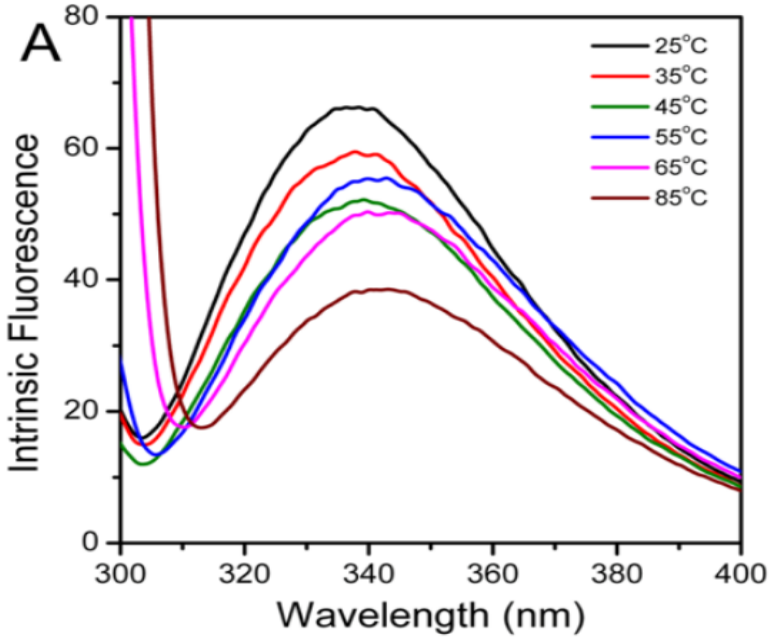
  blood cancers

Fluorescent scanners???



Effect of temperature

Generally, an increase in temperature results in a decrease in the fluorescence quantum yield and the life time because the non-radiative processes related to thermal agitation (collisions with solvent molecules, intramolecular vibrations and rotations, etc.) are more efficient at higher temperatures.



Atomic Spectroscopy

Atomic absorption, along with atomic emission, was first used by Guystav Kirchhoff and Robert Bunsen in 1859 and 1860, as a means for the qualitative identification of atoms. Although atomic emission continued to develop as an analytical technique, progress in atomic absorption languished for almost a century.

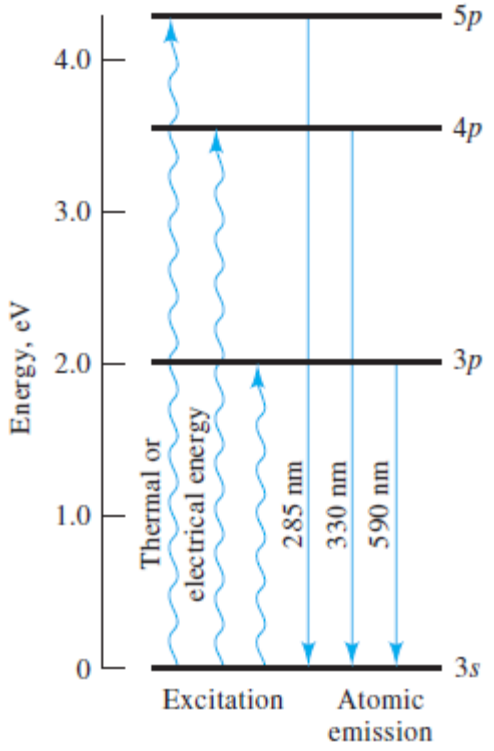
Modern atomic absorption spectroscopy was introduced in 1955 as a result of the independent work of A. Walsh and C. T. J. Alkemade.

Commercial instruments were in place by the early 1960s, and the importance of atomic absorption as an analytical technique was soon evident.

Atomic Spectroscopy

Origins of Atomic Spectra: Emission Spectra

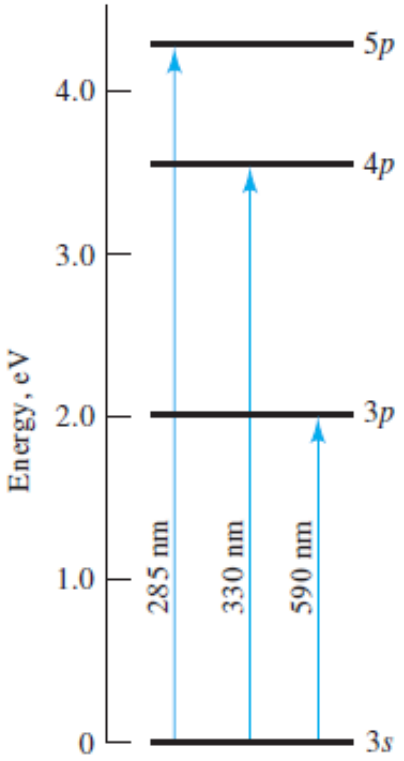
- Before the external energy source is applied, atoms are in their lowest-energy or **ground state**.
- Analyte atoms are excited by heat or electrical energy.
- Atoms are momentarily in a higher-energy or **excited state**.
- External energy promotes the outer electrons from their ground state orbitals to excited-state orbitals.
- After a few nanoseconds, the excited atoms relax to the ground state giving up their energy as photons of visible or ultraviolet radiation.



Atomic Spectroscopy

Origins of Atomic Spectra: Absorption Spectra

An external source of radiation impinges on the analyte vapor. If the source radiation is of the appropriate frequency (wavelength), it can be absorbed by the analyte atoms and promote them to excited states.



Absorption of radiation of 285, 330, and 590 nm excites the single outer electron of sodium from its ground state 3*s* energy level to the excited 3*p*, 4*p*, and 5*p* orbitals, respectively.

After a few nanoseconds, the excited atoms relax to their ground state by transferring their excess energy to other atoms or molecules in the medium.

Atomic Absorption Spectroscopy

Molecular Absorption Spectrophotometer (MAS)

Atomic Absorption Spectrophotometer (AAS)

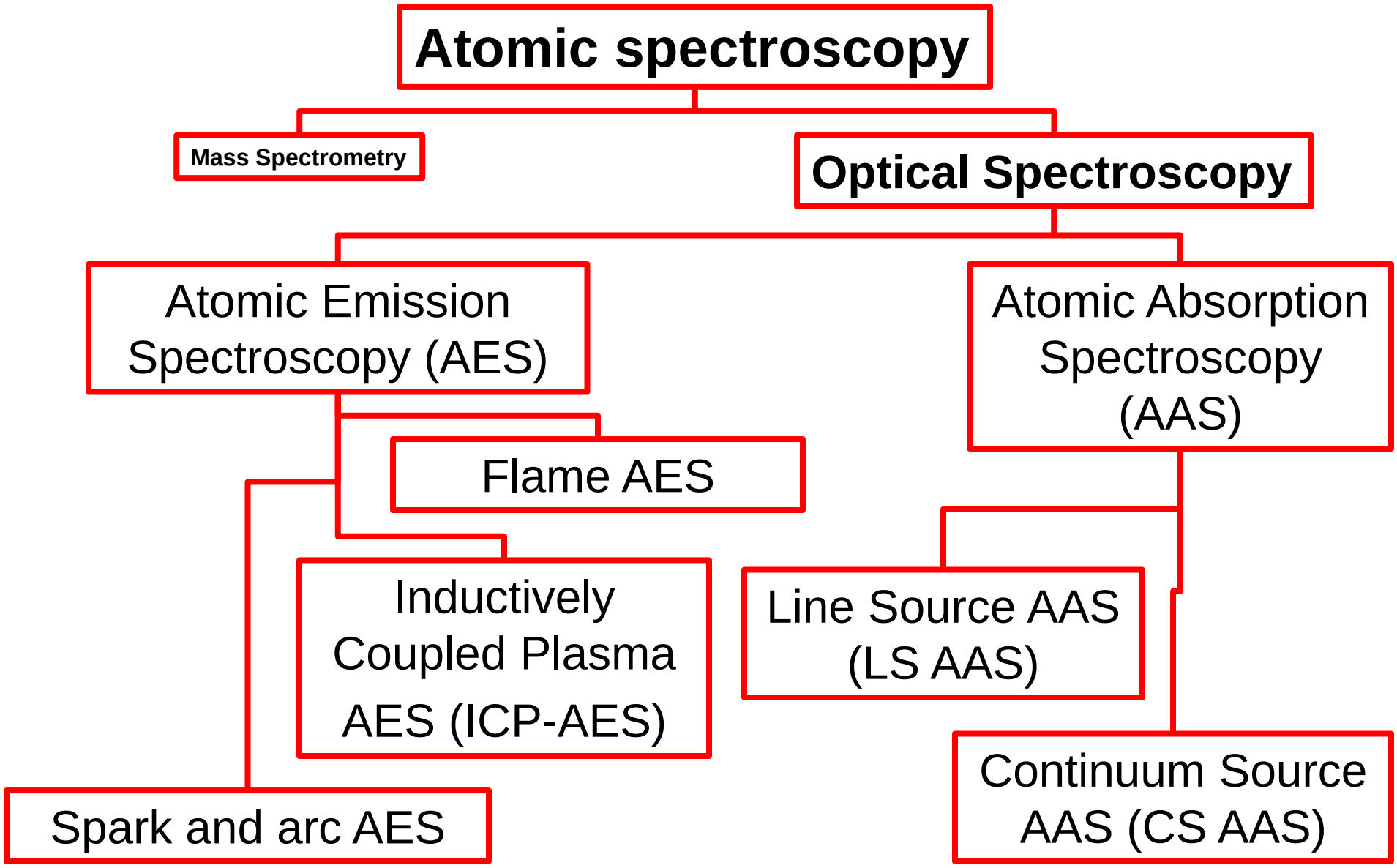
What is the most important difference between MAS and AAS??!

The need to convert the analyte into a free atom

The process of converting an analyte in solid, liquid, or solution form to a free gaseous atom is called:

Atomization

Atomic spectroscopy



Atomic Absorption Spectroscopy

Instrumentation

In most cases, samples containing the analyte undergo some form of sample preparation that leaves the analyte in an organic or aqueous solution.

Two general methods of atomization are used:

1. Flame atomization,
2. Electrothermal atomization.

A few elements are atomized using other methods, like: ICP, DCP, Electric Arc, Electric Spark.

ICP: Inductively Coupled Plasma,

DCP: Direct Current Plasma.

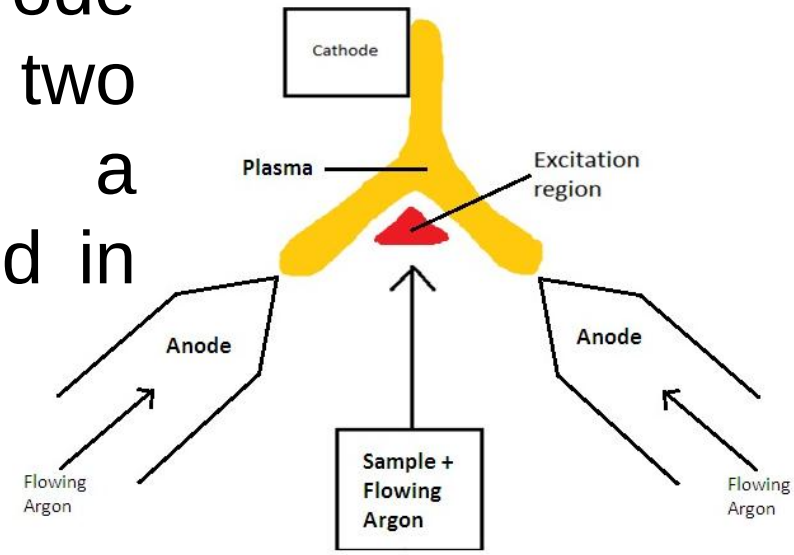
Plasma

plasma is a conducting gaseous mixture containing a significant concentration of ions and electrons.

- **Direct-current plasma (DCP):**

A type of plasma source, with three electrodes to produce a plasma stream.

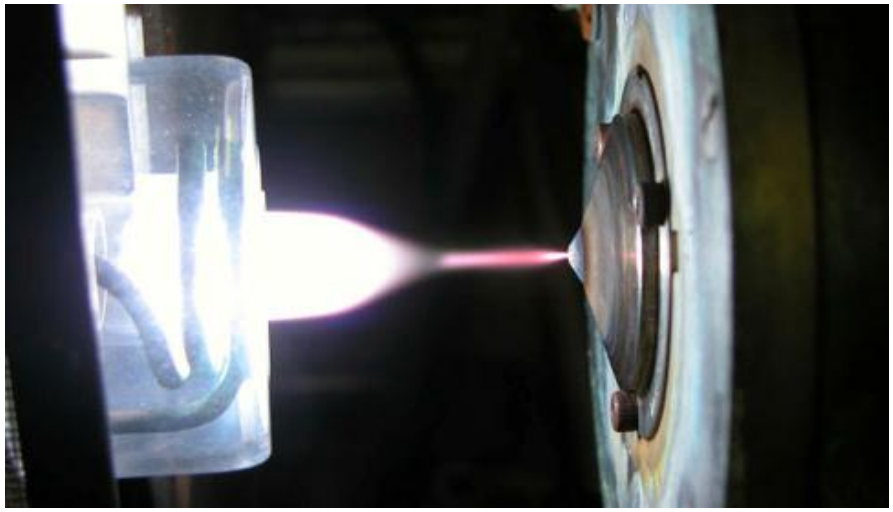
The most common three-electrode DCP apparatus consists of two graphite anode blocks and a tungsten cathode block arranged in an inverted-Y arrangement.

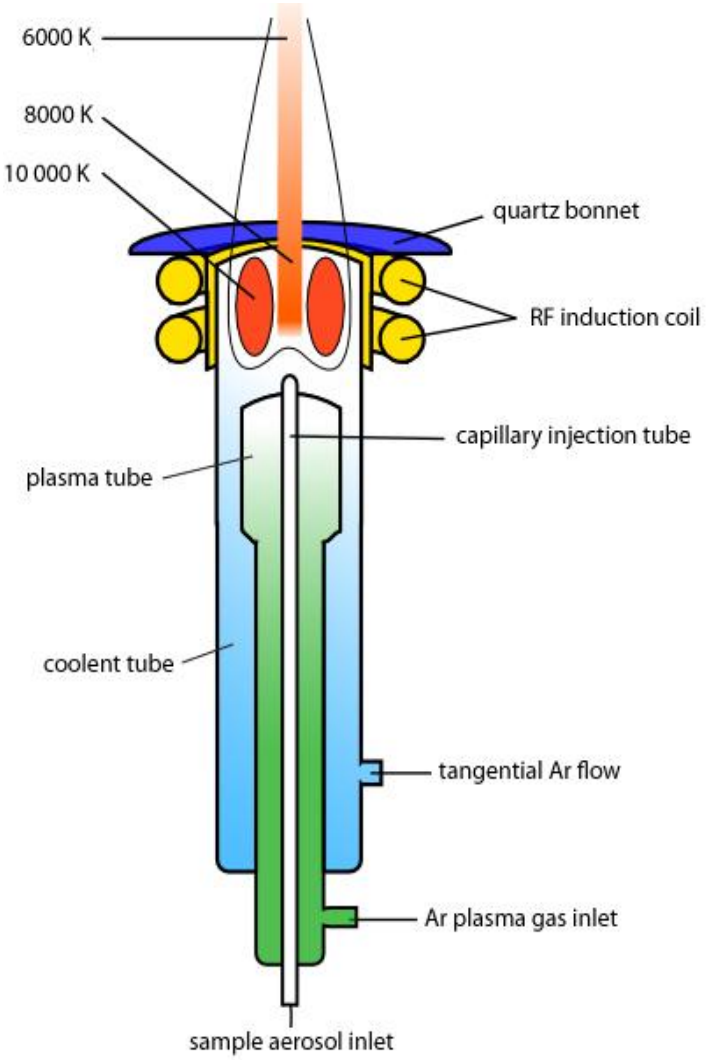


Plasma

❑ Inductively Coupled plasma (ICP):

A type of plasma source in which the energy is supplied by electric currents which are produced by electromagnetic induction.





Distribution between Ground and Excited States

Maxwell–Boltzmann expression

$$\frac{N_e}{N_0} = \frac{g_e}{g_0} e^{-(E_e - E_0)/kT}$$

The populations of ground-state ($\mathbf{N_0}$) and excited-state ($\mathbf{N_e}$),

$\mathbf{g_e}$ and $\mathbf{g_0}$ are the degeneracies of the excited and ground states,

$\mathbf{E_e}$ and $\mathbf{E_0}$ are the energies of the two states,

(E_e = hv, E_0 is usually zero),

k is the Boltzmann constant (1.3805×10^{-16} erg K^{-1}),

T is the absolute temperature.

	N_e/N_0		
Line (nm)	2000 K	3000 K	10,000 K
Na 589.0	9.9×10^{-6}	5.9×10^{-4}	2.6×10^{-1}
Ca 422.7	1.2×10^{-7}	3.7×10^{-5}	1.0×10^{-1}
Zn 213.8	7.3×10^{-15}	5.4×10^{-10}	3.6×10^{-3}

Classification of Atomic Spectroscopic Methods

Atomization Method	Typical Atomization Temperature, °C	Types of Spectroscopy	Common Name and Abbreviation
Inductively coupled plasma	6000–8000	Emission	Inductively coupled plasma atomic emission spectroscopy, ICPAES
		Mass	Inductively coupled plasma mass spectrometry, ICP-MS (see Chapter 29)
Flame	1700–3150	Absorption	Atomic absorption spectroscopy, AAS
		Emission	Atomic emission spectroscopy, AES
		Fluorescence	Atomic fluorescence spectroscopy, AFS
Electrothermal	1200–3000	Absorption	Electrothermal AAS
		Fluorescence	Electrothermal AFS
Direct-current plasma	5000–10,000	Emission	DC plasma spectroscopy, DCP
Electric arc	3000–8000	Emission	Arc-source emission spectroscopy
Electric spark	Varies with time and position	Emission	Spark-source emission spectroscopy
		Mass	Spark-source mass spectroscopy

Types of Flames Used in Atomic Spectroscopy

Fuel and Oxidant	Temperature, °C
*Gas/Air	1700–1900
*Gas/O_2	2700–2800
H_2/air	2000–2100
H_2/O_2	2500–2700
C_2H_2/air	2100–2400
C_2H_2/O_2	3050–3150
C_2H_2/N_2O	2600–2800

*Propane or natural gas

Determination Limits (ng/mL):Atomic Spectroscopy

Element	Flame AA	Electrothermal AA†	Flame Emission	ICP Emission	ICPMS
Ag	3	0.02	20	0.2	0.003
Al	30	0.2	5	0.2	0.06
Ba	20	0.5	2	0.01	0.002
Ca	1	0.5	0.1	0.0001	2
Cd	1	0.02	2000	0.07	0.003
Cr	4	0.06	5	0.08	0.02
Cu	2	0.1	10	0.04	0.003
Fe	6	0.5	50	0.09	0.45
K	2	0.1	3	75	1
Mg	0.2	0.004	5	0.003	0.15
Mn	2	0.02	15	0.01	0.6
Mo	5	1	100	0.2	0.003
Na	0.2	0.04	0.1	0.1	0.05
Ni	3	1	600	0.2	0.005
Pb	5	0.2	200	1	0.007
Sn	15	10	300	1	0.02
V	25	2	200	8	0.005
Zn	1	0.01	200	0.1	0.008

Interferences in AAS

1- Spectral Interferences

- Undissociated molecules, and Matrix effect.

Solution: background absorption

2- AAS Ionization Interference

- Ca/Na interference : A positive measurement error.

3- Refractory Compound Formation:

(resistant to decomposition by heat)

- (Ca); presence of phosphate: formation of calcium pyrophosphate, $Ca_2P_2O_7$.
- Al, Ti, V, Mo: Oxide formation.

Interferences in AAS

4- Physical Interferences:

Parameters affecting the rate of sample uptake in the burner and the atomization efficiency:

1. Variations in the gas flow rates,
2. Variation in sample viscosity due to temperature or solvent variation,
3. High and variable solids content,
4. Changes in the flame temperature.