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4) Spectroscopy

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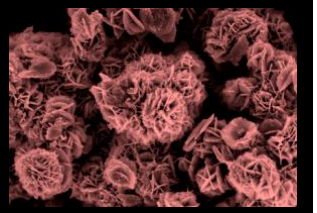
Atomic Spectroscopy

- Atomic Emission
- Atomic Absorption
- Atomic Fluorescence
- Use of X-rays

Molecular Spectroscopy

- Fourier Transform Infra-red (FT-IR)
- Organic UV-Vis Absorption
- Effect of Solvent
- Inorganic UV-Vis Absorption
- Qualitative Analysis
- Quantitative Analysis
- Molecular Fluorescence
- Equipment for Molecular Spectroscopy

Syllabus Recap





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MOLECULAR SPECTROSCOPY

CEB 4032: ANALYTICAL CHEMISTRY
CFB3032: ANALYTICAL INSTRUMENTATION

TS. CHM. DR. MOHD DZUL HAKIM WIRZAL

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Outline

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- Fourier Transform Infrared (FTIR)
- Organic UV-Vis Absorption
- Effect of Solvent
- Inorganic UV-Vis Absorption
- Qualitative Analysis
- Quantitative Analysis
- Molecular Fluorescence
- Equipment for Molecular Spectroscopy

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Learning Outcomes

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At the end of the chapter:

1) Principles and application of molecular spectroscopy including UV-Vis, FTIR and Fluorescence.

2) Determination of organic, inorganic or biochemical compounds in the unknown sample using molecular spectroscopy .

3) Qualitative and Quantitative analyses using UV-Vis and FT-IR.

4) Instruments for molecular spectroscopy including UV-Vis, FTIR and Fluorescence.

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Introduction

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- Molecular spectroscopy based on ultraviolet, visible and infrared radiation is widely used for the identification and determination of many inorganic, organic and biochemical species.
- Molecular UV/Vis absorption spectroscopy is used primarily for quantitative analysis and is probably more extensively applied in chemical and clinical laboratories.
- Infrared absorption spectroscopy is a powerful tool for determining the structure of both organic and inorganic compounds.

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Fourier Transform Infra-Red (FT-IR)

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One of the premier techniques for qualitative analysis. In IR region, absorption of radiation give information:

- Identity of compounds
- Presence or absence of functional groups (C=O,C=C,C-H, C≡C or O-H)
- Structure of molecules.

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Fourier Transform Infra-Red (FT-IR)

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- IR radiation involves transitions among the vibrational and rotational energy levels → lowest excited energy levels of molecules.

- Infrared are not energetic enough to introduce electronic transitions

- Change the vibrational or rotation motion of the molecule.

Recall: Molecular Absorption

- The energy *E* associated with the bands of a molecule is made up of three components.

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

Rotational → very low energy (low wavelength, microwave or far-infrared region)

Vibrational →requires higher energies (near-infrared region)

Electronic → require still higher energies (visible and ultraviolet regions)

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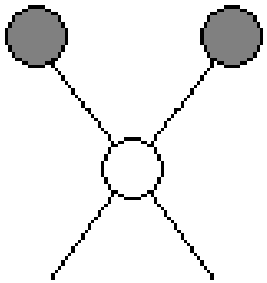
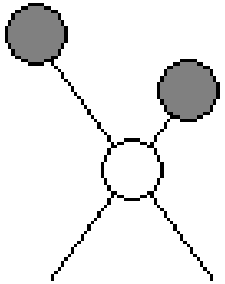
Stretching vibrations

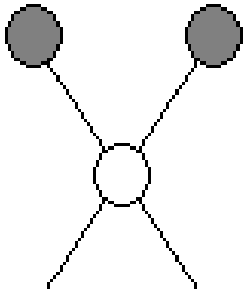


Symmetric



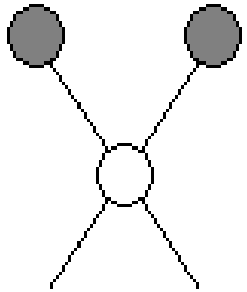
Asymmetric

Bending vibrations



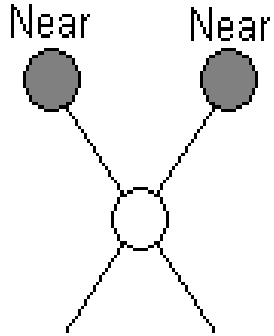
In-plane rocking

Asymmetric bending

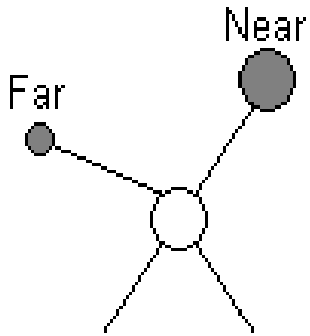


In-plane scissoring

Symmetric bending



Out-of-plane wagging



Out-of-plane twisting

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Infrared Regions

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i) Near IR -4000-14000 cm^{-1}

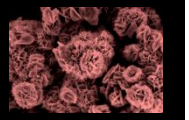
- for routine quantification determination of certain species, such as water, CO_2, sulfur, HCs, amine nitrogen.

ii) Mid IR - 670-4000 cm^{-1}

- most widely used region
- determining the structure of organic and biochemical species

iii) Far IR- < 650 cm^{-1}

- determination of the structure of inorganic and metal-organic species.





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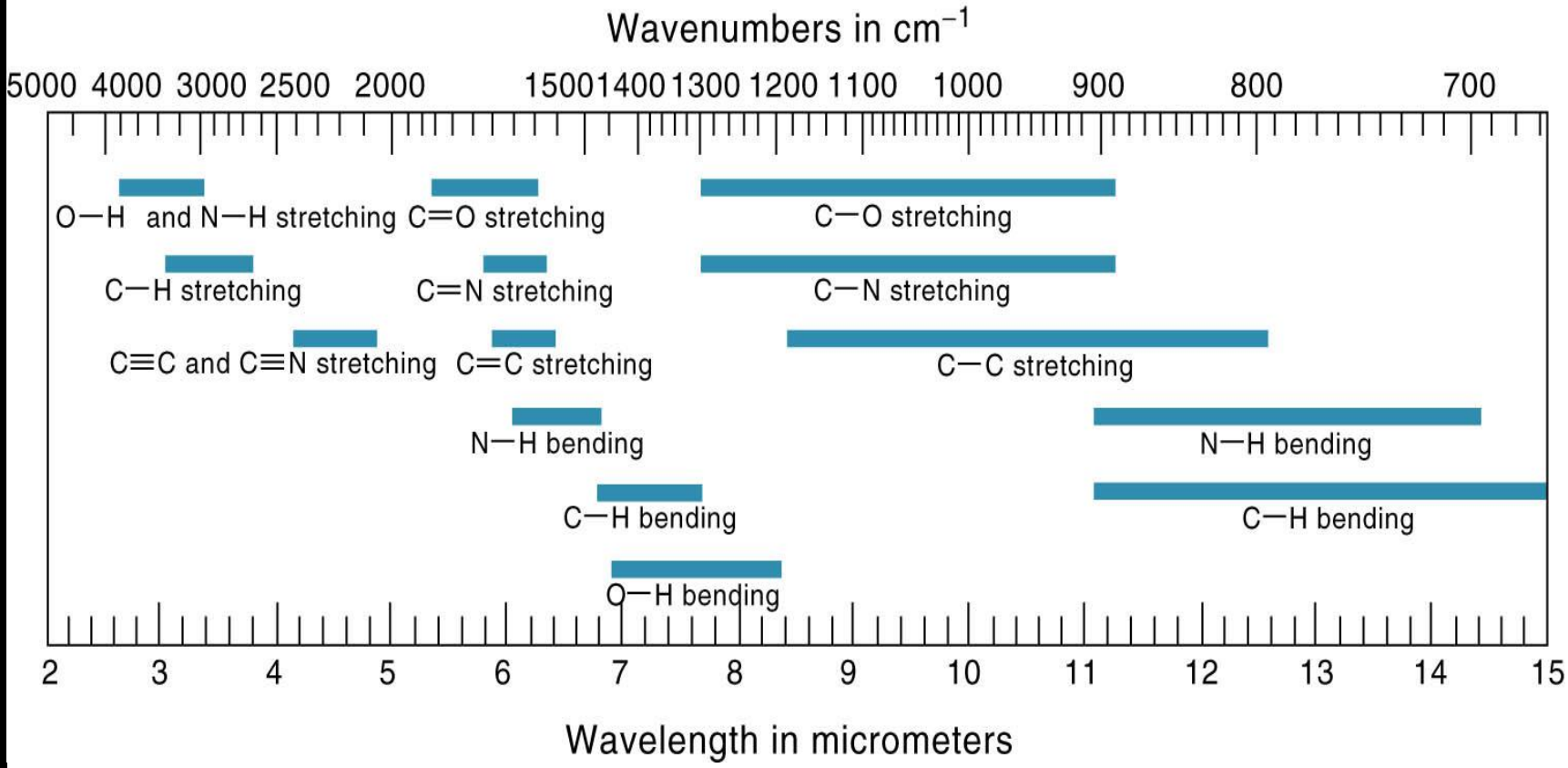
Recall:



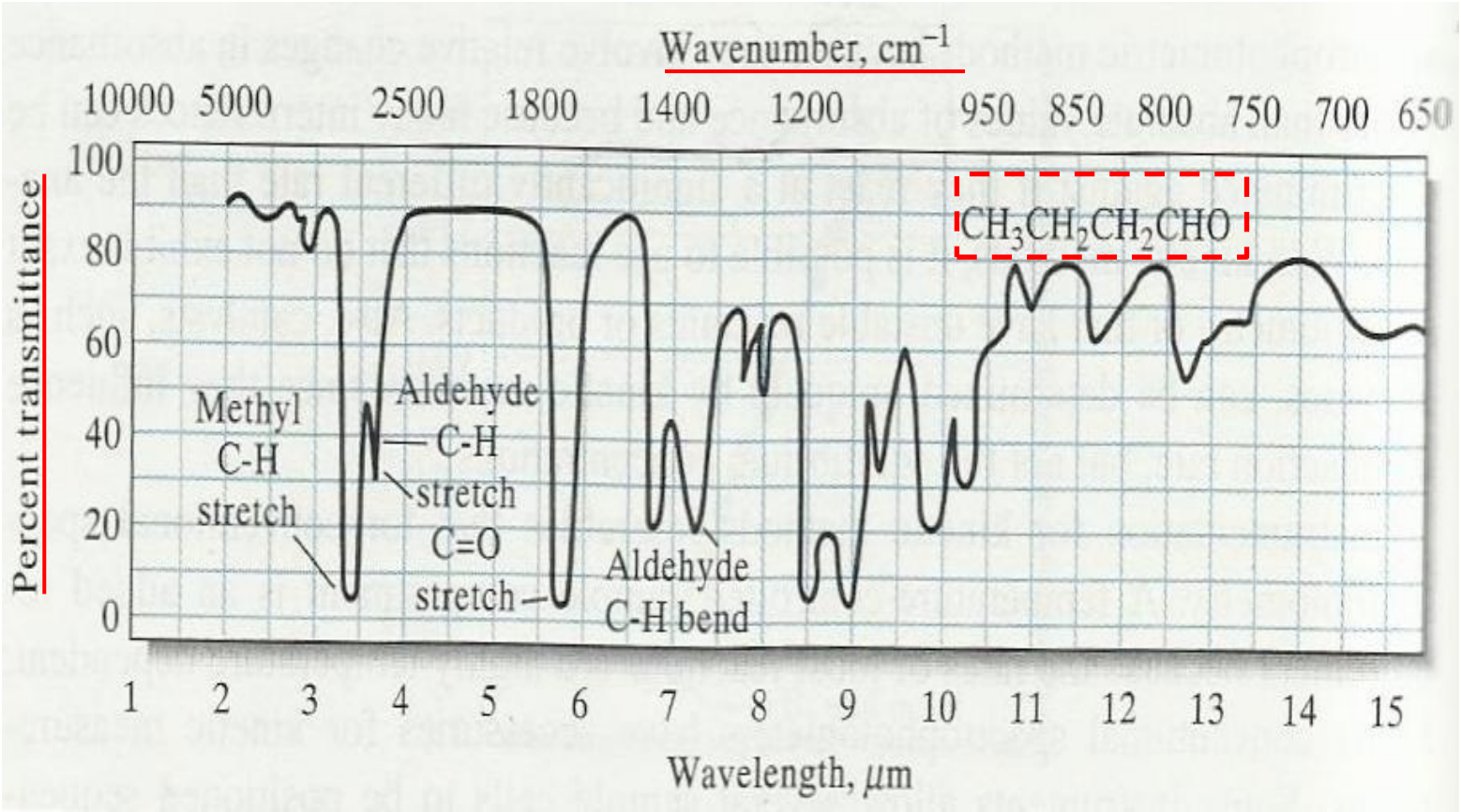
Simple correlations of group vibrations to regions of infrared absorption

Typical functional groups that can be identified include alcohol, hydroxyl, ester carbonyl, olefin and aromatic unsaturated hydrocarbon group.

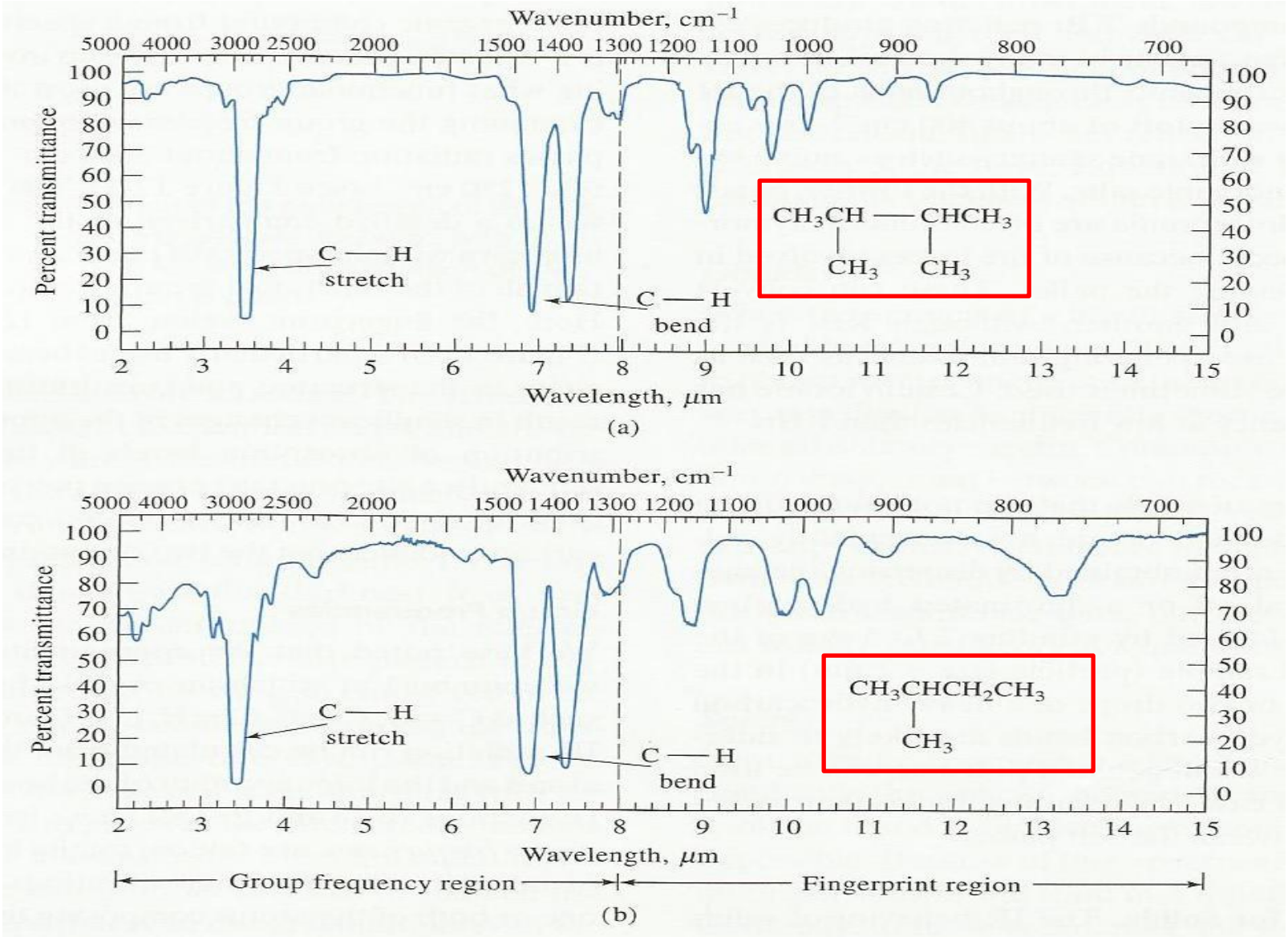
Abbreviation Table of Group Frequencies for Organic Functional Groups

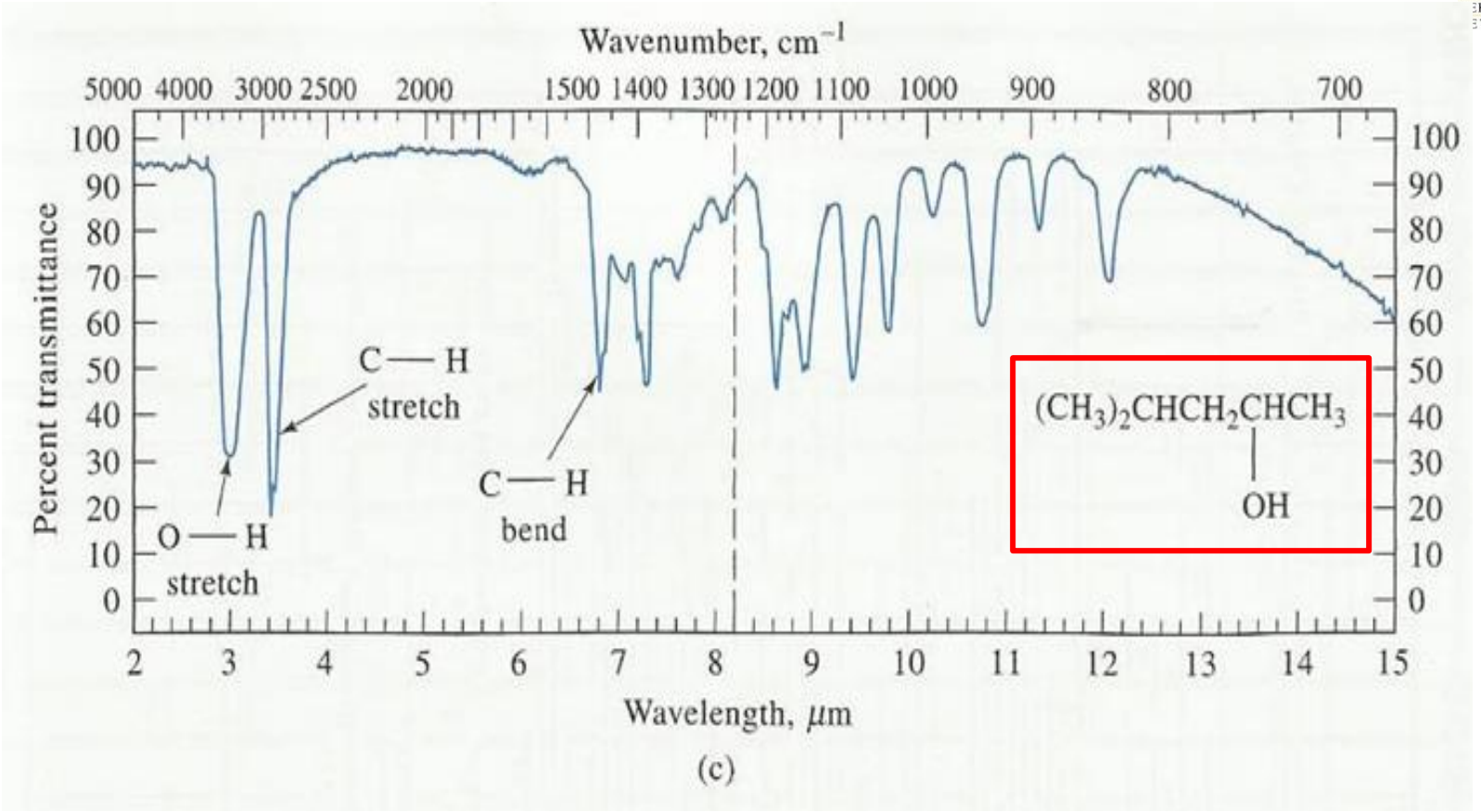
Bond	Type of Compound	Frequency Range, cm^{-1}	Intensity
C—H	Alkanes	2850–2970	Strong
		1340–1470	Strong
C—H	Alkenes $\left(\rangle C{=}C\langle^{H}\right)$	3010–3095	Medium
		675–995	Strong
C—H	Alkynes (—C≡C—H)	3300	Strong
C—H	Aromatic rings	3010–3100	Medium
		690–900	Strong
O—H	Monomeric alcohols, phenols	3590–3650	Variable
	Hydrogen-bonded alcohols, phenols	3200–3600	Variable, sometimes broad
	Monomeric carboxylic acids	3500–3650	Medium
	Hydrogen-bonded carboxylic acids	2500–2700	Broad
N—H	Amines, amides	3300–3500	Medium
C=C	Alkenes	1610–1680	Variable
C=C	Aromatic rings	1500–1600	Variable
C≡C	Alkynes	2100–2260	Variable
C—N	Amines, amides	1180–1360	Strong
C≡N	Nitriles	2210–2280	Strong
C—O	Alcohols, ethers, carboxylic acids, esters	1050–1300	Strong
C=O	Aldehydes, ketones, carboxylic acids, esters	1690–1760	Strong
NO_2	Nitro compounds	1500–1570	Strong
		1300–1370	Strong

Example: IR spectrum for *n*-butanal (*n*-butyraldehyde)

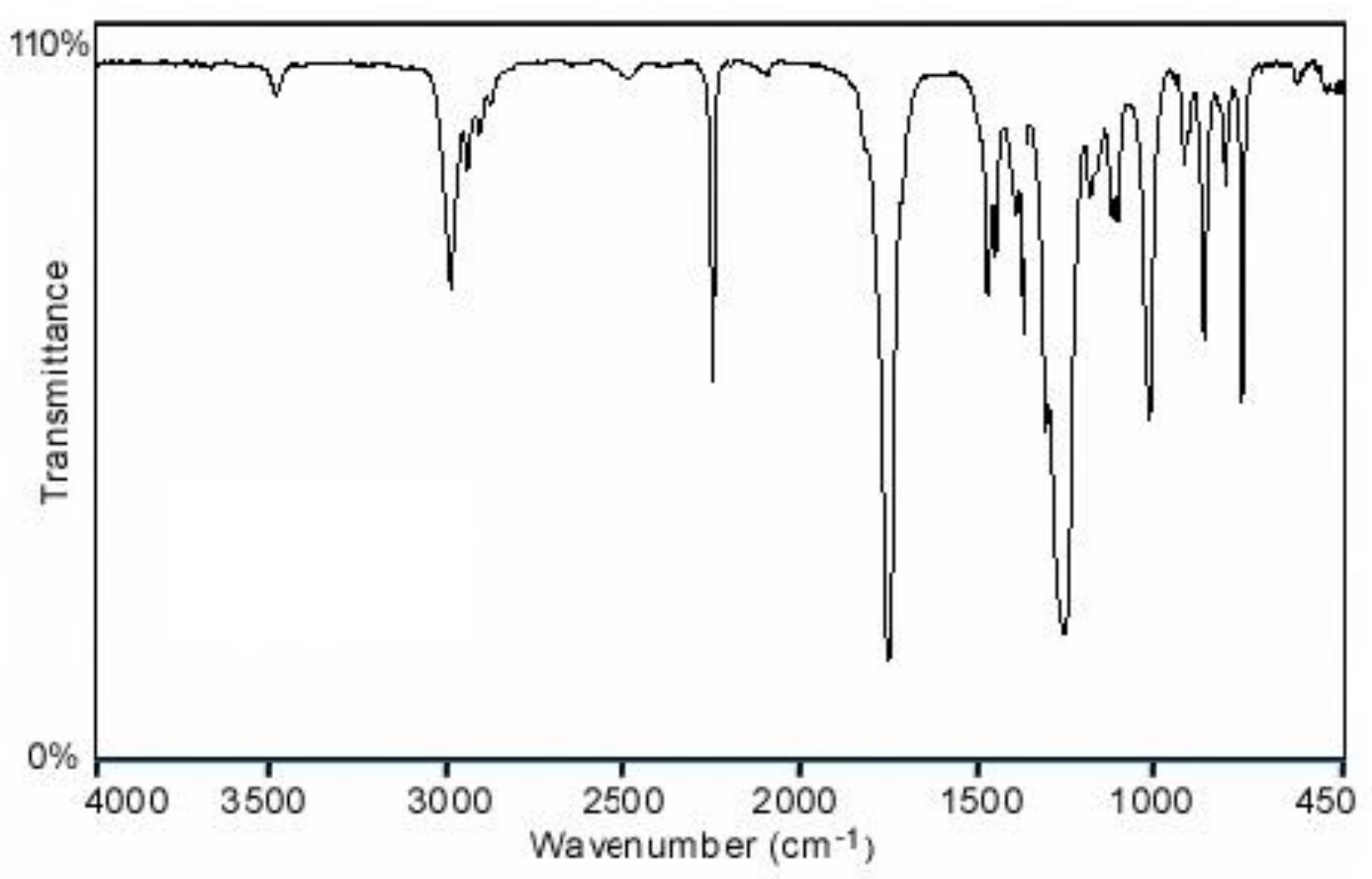


Examples: IR spectrum for other compounds




Wavenumber, cm⁻¹
5000 4000 3000 2500 2000 1500 1400 1300 1200 1100 1000 900 800 700
Percent transmittance
100 90 80 70 60 50 40 30 20 10 0
C — H
stretch
C — H
bend
O — H
stretch
(CH3)2CHCH2CHCH3
OH
2 3 4 5 6 7 8 9 10 11 12 13 14 15
Wavelength, μm
(c)

Identify the molecular structure based on this IR spectrum: Example 1



Given: Molecular formula= $C_4H_5O_2N$

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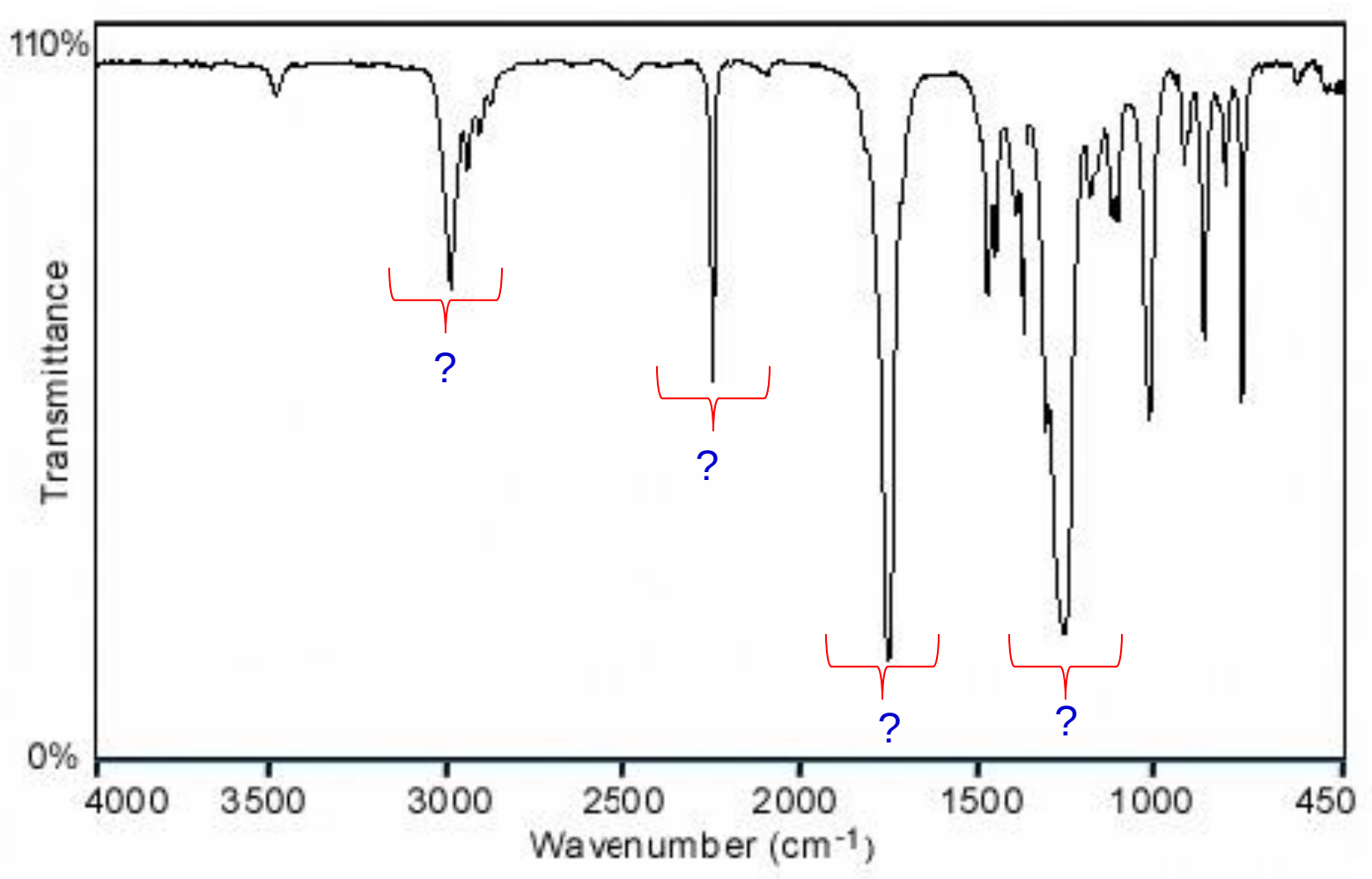
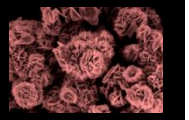
Five Important Steps in identifying the molecular structure of the sample from FT-IR Spectrum

1) Check molecular formula given
2) Identify the peaks (wavenumber range)→ possible vibration group by referring to the correlation table
3) Draw the possible structure
4) Check the balance of the chemical bonding.
5) The possible structure should contain the vibration groups in Step 2

Identify the molecular structure based on this IR spectrum: Example 1



Molecular formula: $C_4H_5O_2N$





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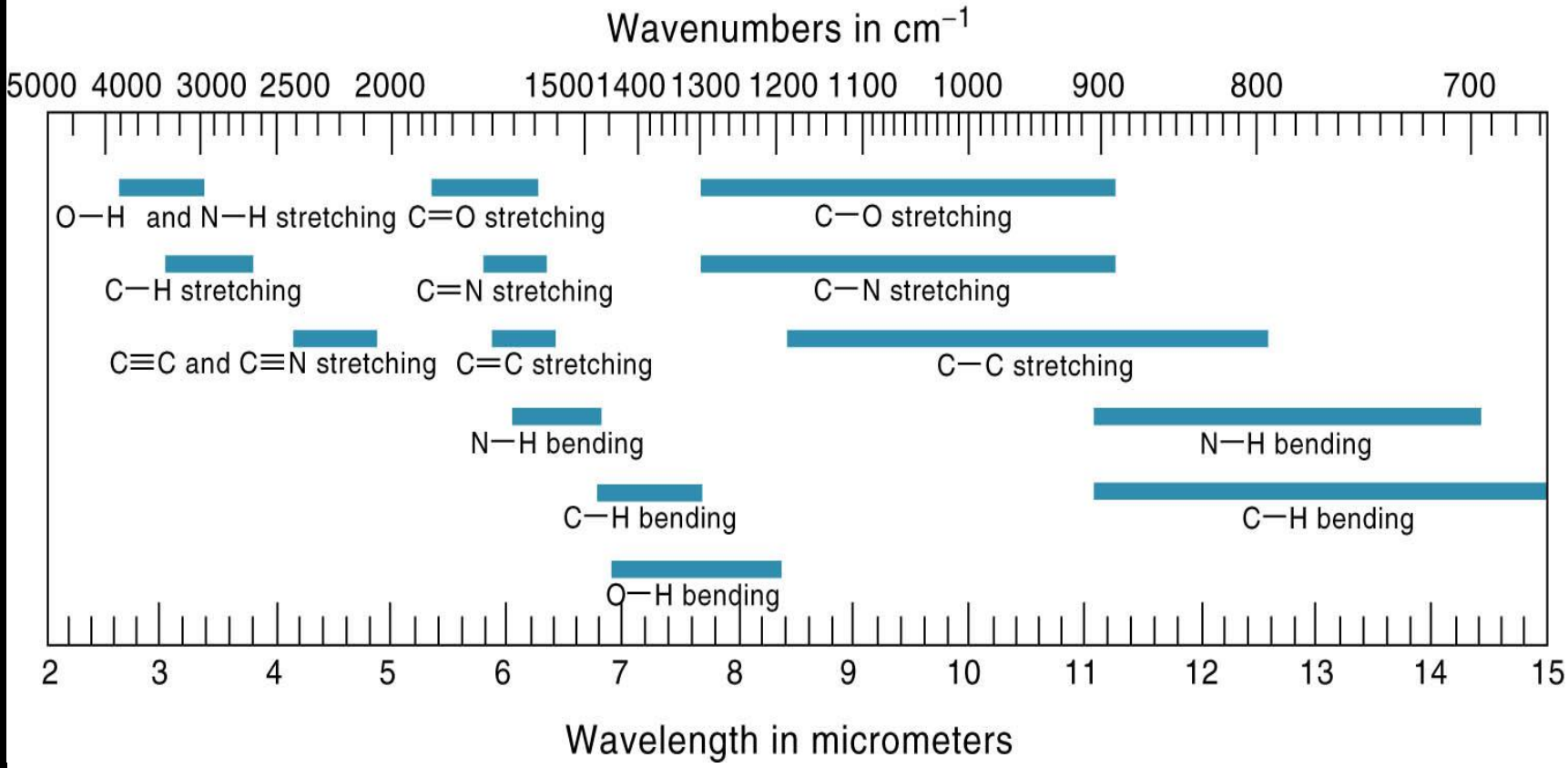
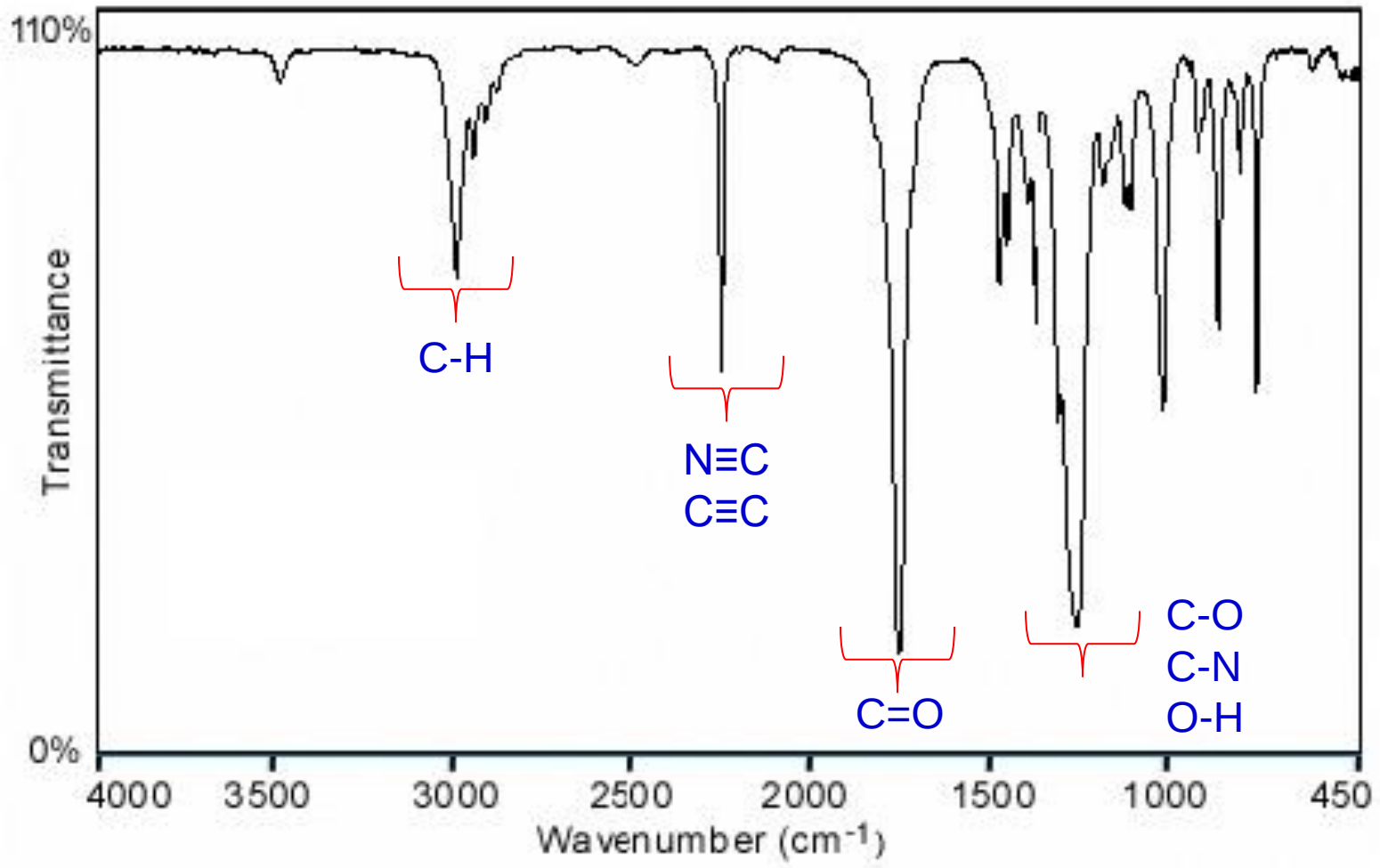
Recall:



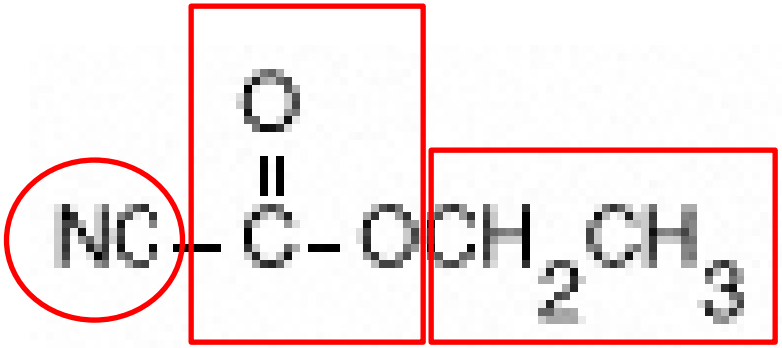
Simple correlations of group vibrations to regions of infrared absorption

Typical functional groups that can be identified include alcohol, hydroxyl, ester carbonyl, olefin and aromatic unsaturated hydrocarbon group.

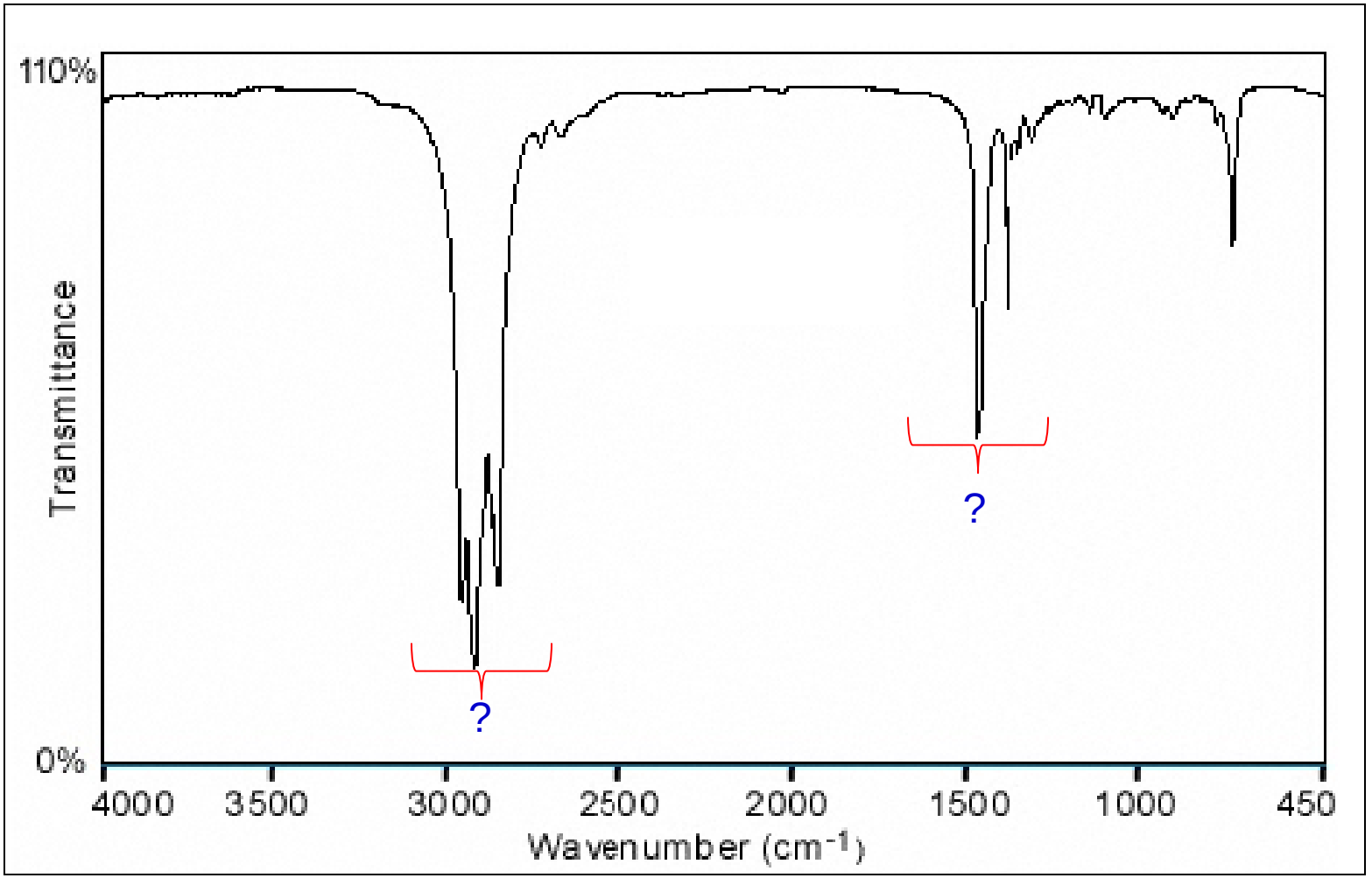
Identify the major peaks



Answer

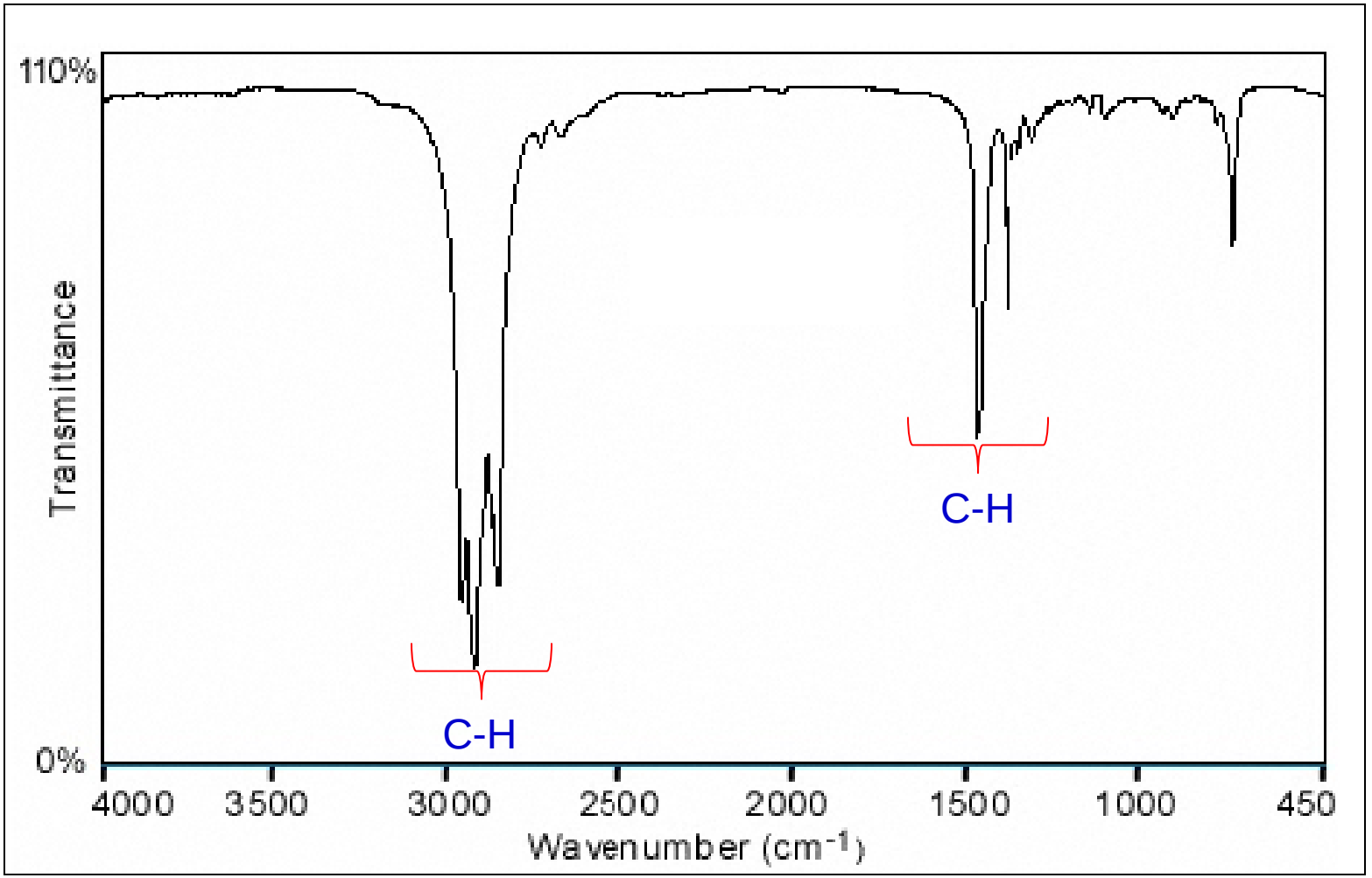


Example 2



Molecular formula: $C_{10}H_{22}$

Identify the major peaks



Molecular formula: $C_{10}H_{22}$

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Answer

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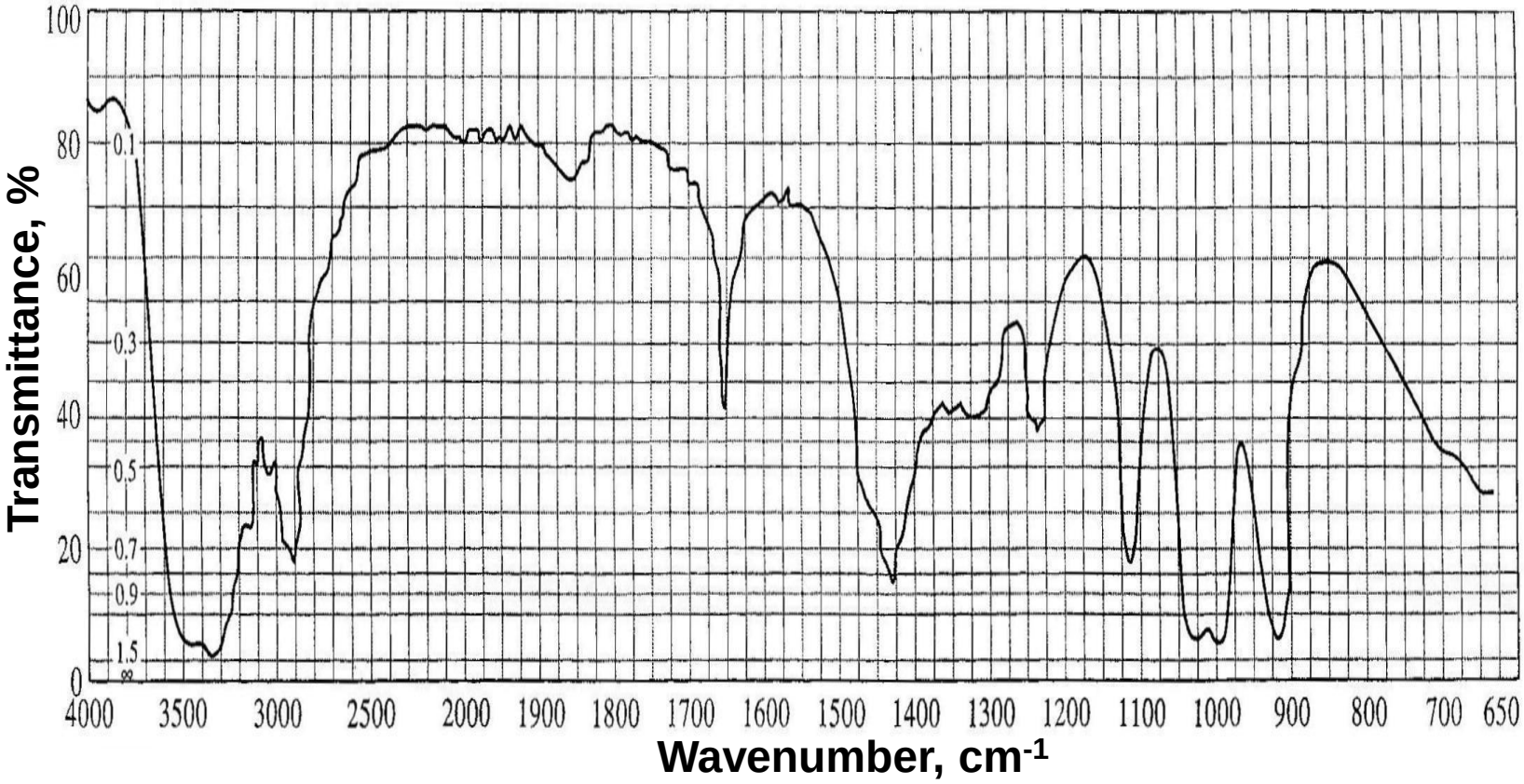
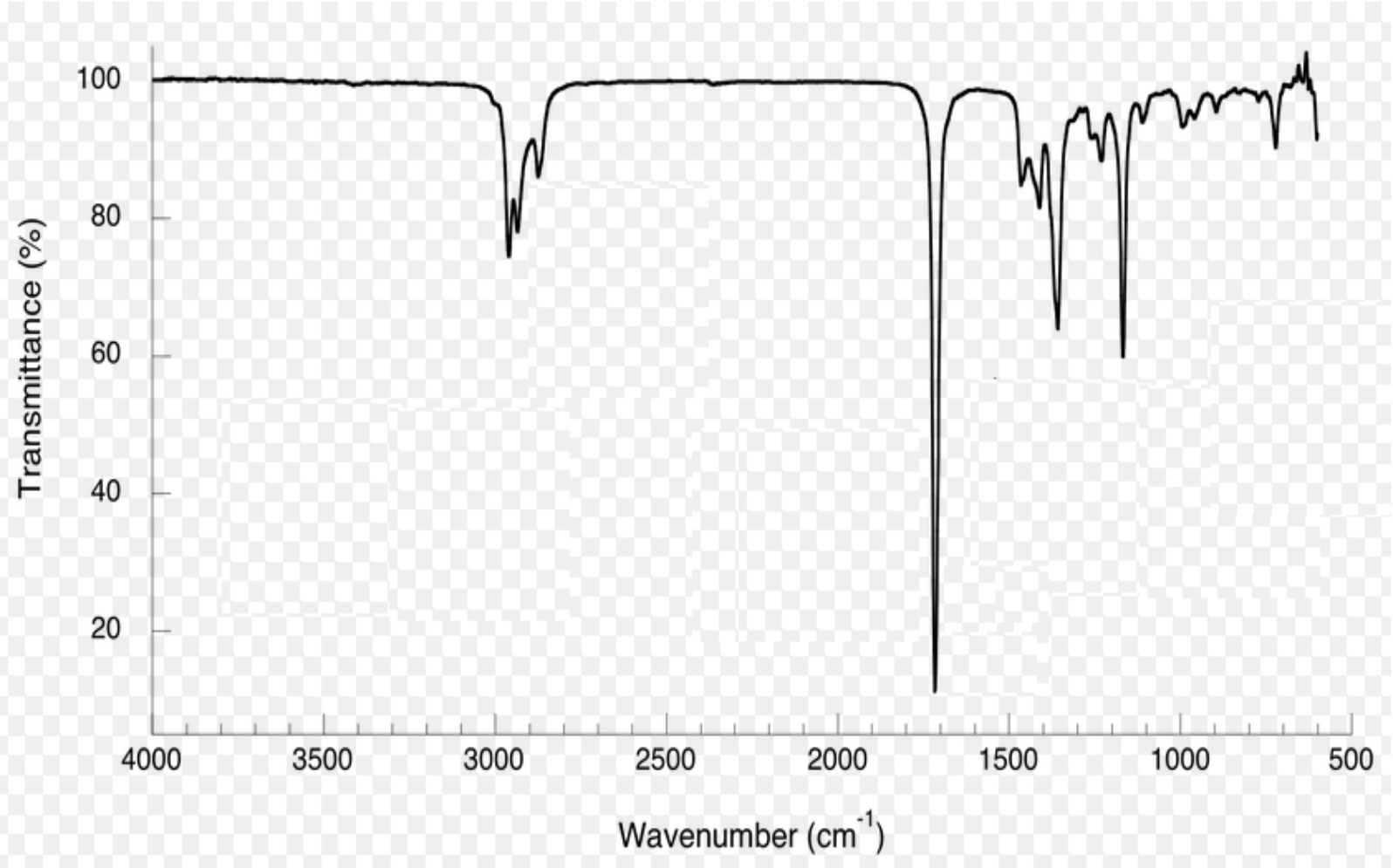
$CH_3\text{-}(CH_2)_8\text{-}CH_3$

Example 3



Given: Molecular formula = C_3H_6O, alcohol

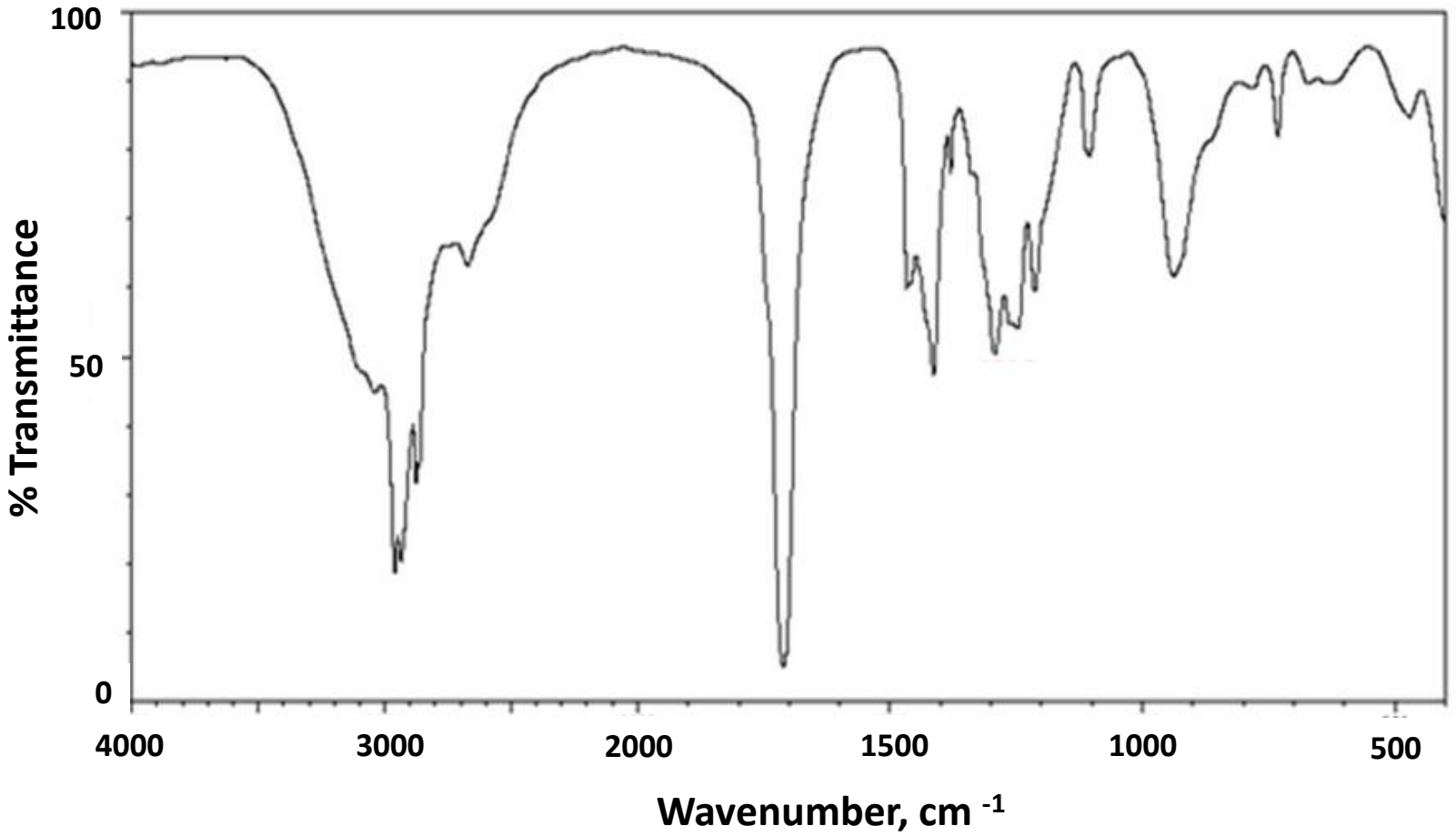
Example 4



Given: Molecular formula = $C_6H_{12}O$

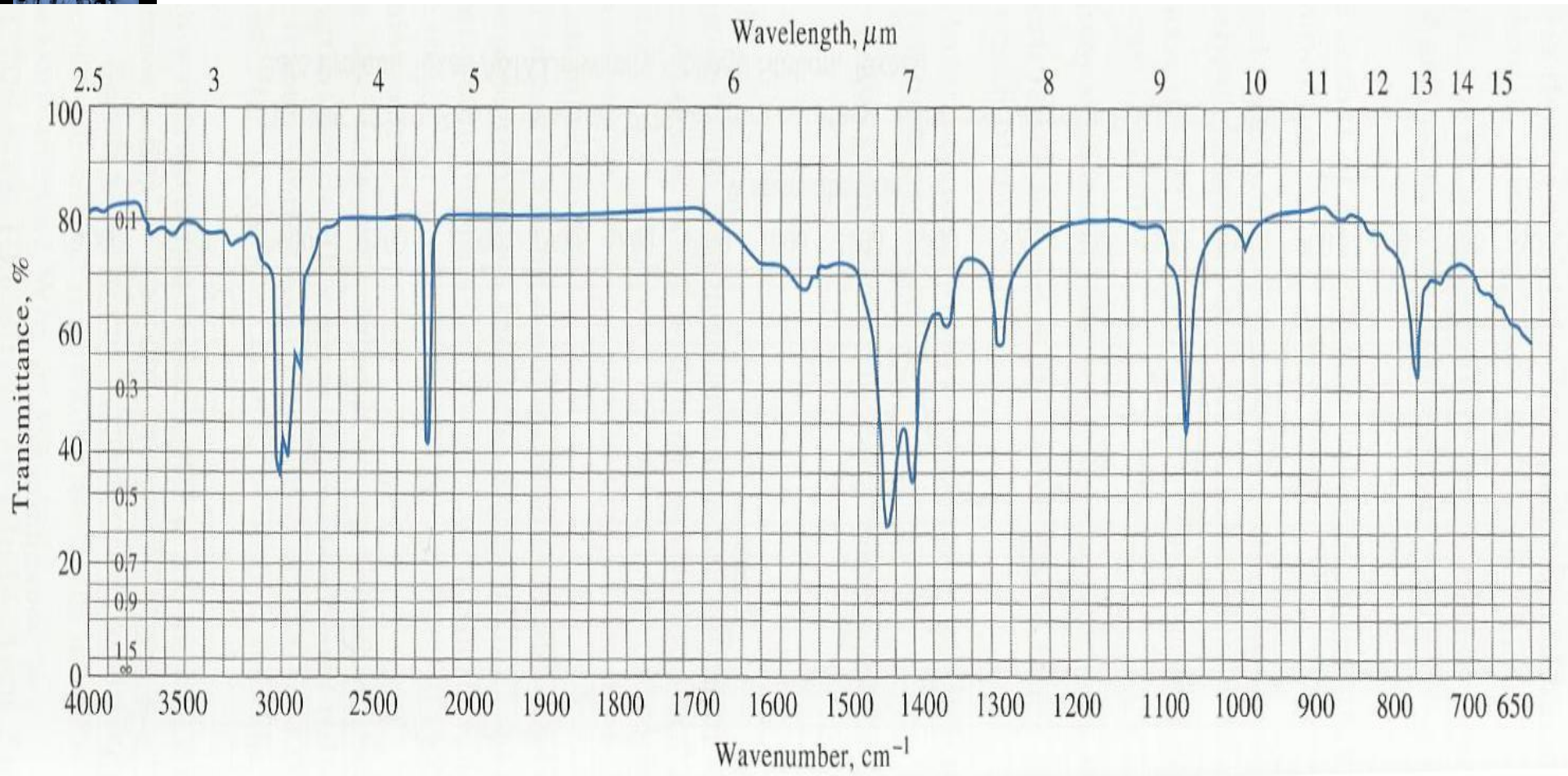
Example 5

The following figure shows the FTIR spectrum of an acid compound with the formula of $C_6H_{12}O_2$



Example 6

The spectrum below is that of a nitrogen-containing substance and has a molecular weight of about 55. Sketch the possible molecular structure of this substance?



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Outline

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- Fourier Transform Infrared (FTIR)
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Ultraviolet and Visible (UV/Vis) Absorption Spectroscopy

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- Are widely used for all quantitative analysis in chemical, environmental, forensic and clinical laboratories.
- The region of spectrum: UV→ 185-400 nm, visible → 400-700 nm.
- The absorption of UV/Vis generally results from excitation of electrons.
- Valuable for identifying functional groups in a molecule or compound containing absorbing group.

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UV/Vis Spectrum

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- UV/Vis spectrometers collate the data over the required range and generate the spectrum of the compound under analysis as a graph representing the transmittance/absorbance as a function of wavelength, unit → cm^{-1}.

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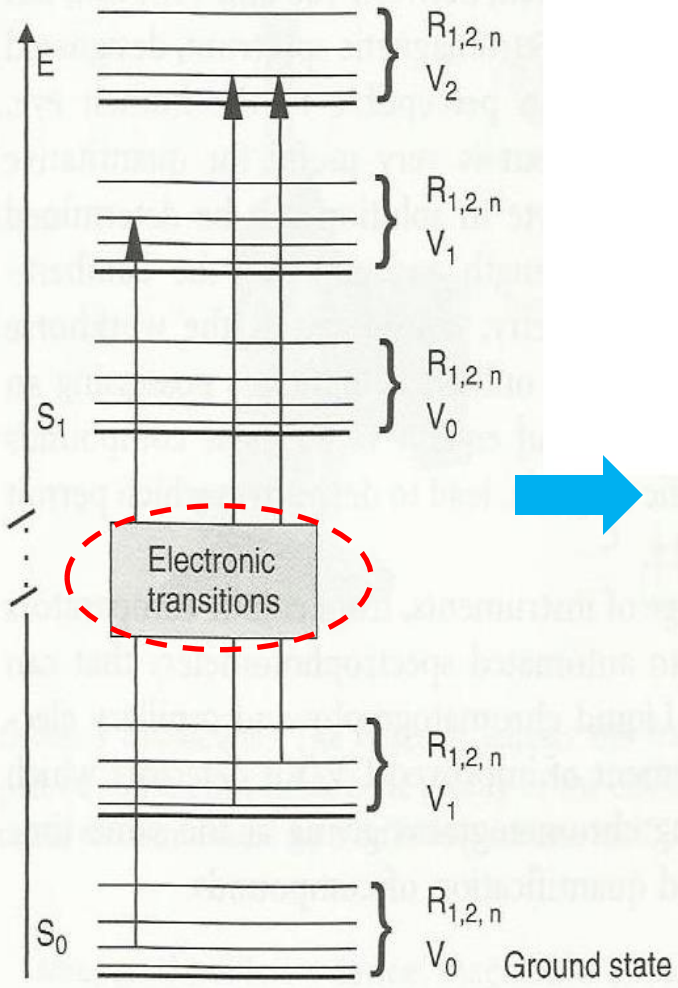

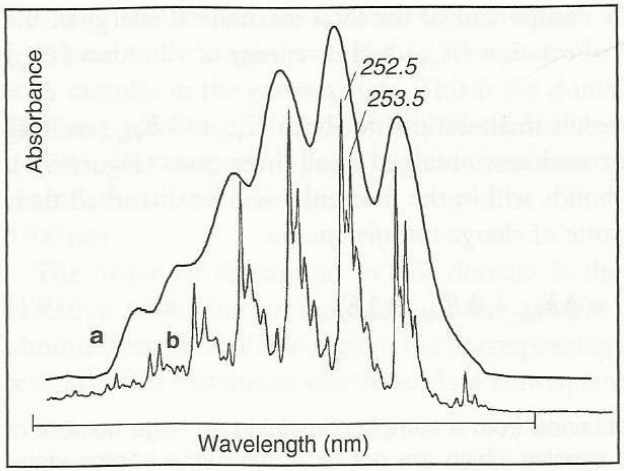

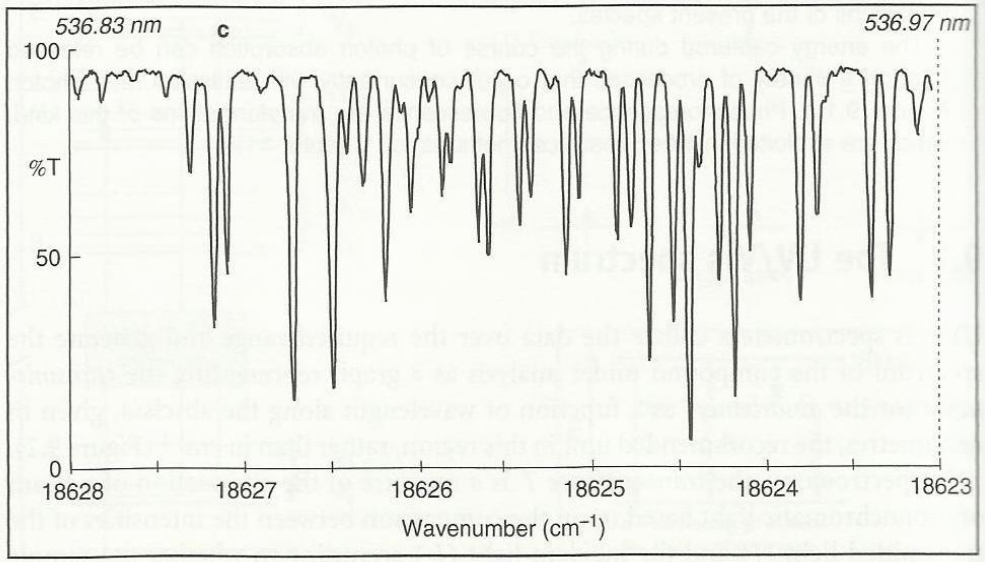

Three different aspects of UV/Vis spectra

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Molecular Spectroscopy

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- Fourier Transform Infrared (FTIR)
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Organic UV/Vis Absorption: Kind of Transitions

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- All organic compound contain valence electrons that can be excited to higher energy level.→ transitions of electrons.
- Electron involved in UV/Vis absorption can be classified into three different types.
- (1) Covalent single bond electrons (*σ* or sigma) → posses too high excitation energy to contribute to absorption of UV/ Vis radiation, (e.g. single valence bonds in saturated HC,-CH_2-CH_2-)
- (2)Paired nonbonding outer shell electron (*n*) →those on N, O, S and halogens, which can be excited by UV/Vis radiation.

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- (3) Electron in pi orbital (π) → double or triple bonds. These electrons are most readily excited and are responsible for a majority of electronic spectra in the UV/Vis regions.

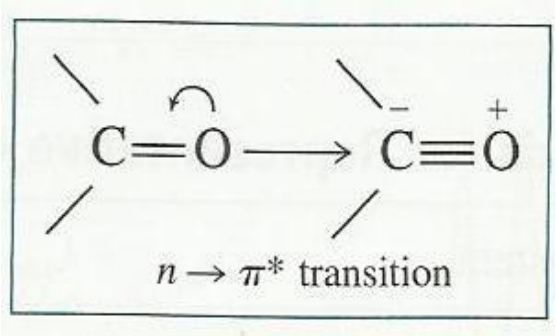
- A molecule also possessed normally unoccupied orbitals called antibonding orbitals → these correspond to excited-state energy levels and are either σ^* or π^* orbitals.

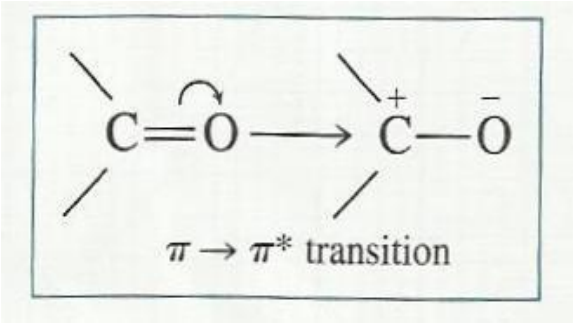
- Therefore, absorption of radiation results in an electronic transition to an antibonding orbitals.

σ	bonding
π	bonding
n	bonding
σ*	anti-bonding
π*	anti-bonding

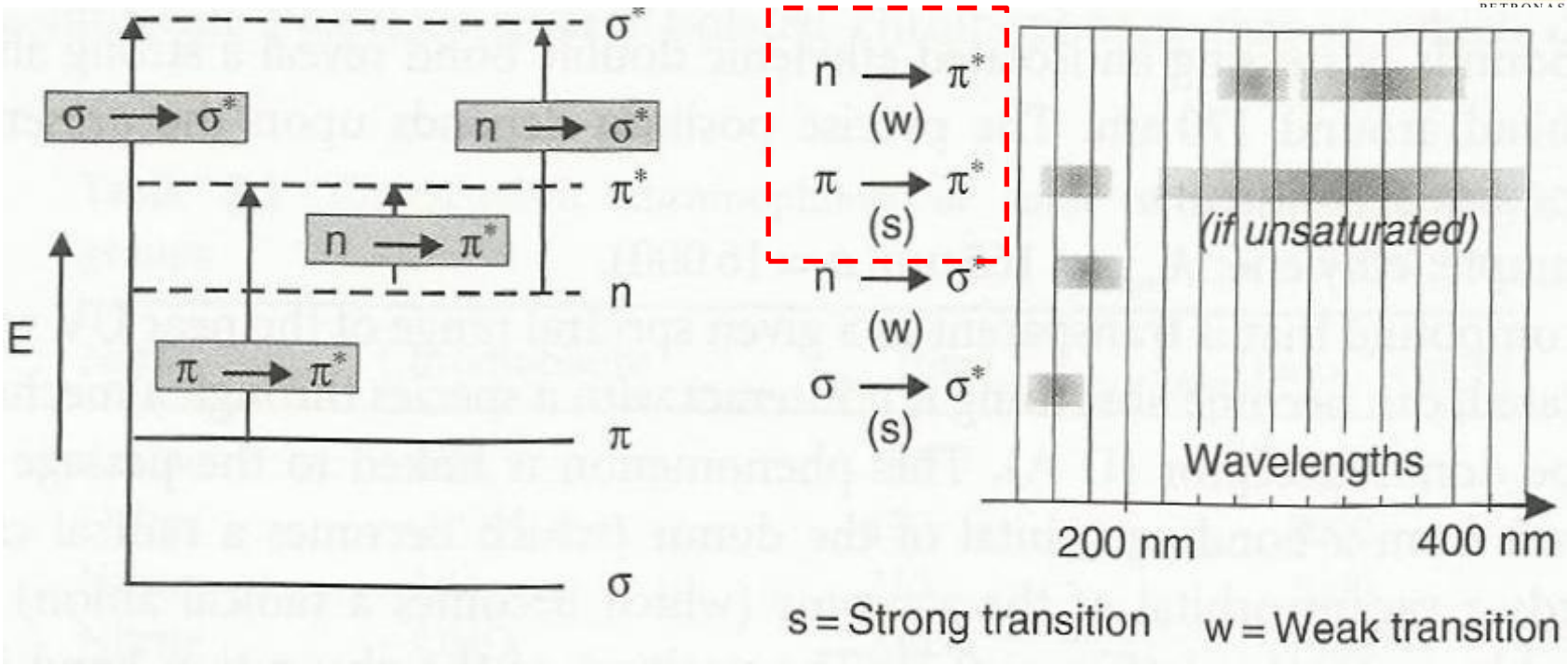
- The most common transitions are from π or n to antibonding π^* orbitals, and these are represented by $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, indicating a transition to an excited π^* stage.

- The nonbonding n electron can also be excited , at a very short wavelengths, to an antibonding σ^* stage: $n \rightarrow \sigma^*$, but occur at wavelength less than 200 nm.





Example transitions occur in ketone



Comparison of the transitions met most frequently with simple organic compound

Probability: $\pi \rightarrow \pi^*$ > $n \rightarrow \pi^*$ > $n \rightarrow \sigma^*$ > $\sigma \rightarrow \sigma^*$

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1) σ → σ* transition

- appears in the far UV, λ_{max} <150 nm
- requires a significant energy.
- adsorption corresponding to breaking of saturated carbon: C-C, C-H, C-O, C-X and etc.
- not observed in a normal UV/Vis work.
- Example: Hexane (gas state) : λ_{max} = 135 nm

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2) n → σ* transition

- promotion of *n* electron from an atom of O, N, S, Cl to an σ*.
- Adsorption range: 150-250 nm.
- Example: 180 nm for alcohols, 190 nm for ethers or halogen derivatives and in the region of 220 nm for amines.

Example	λ_{max}, nm	ε_{max}
H_2O	167	1480
CH_3OH	184	150
CH_3Cl	215	140
CH_3NH_2	227	600
$(CH_3)_2S$	229	140

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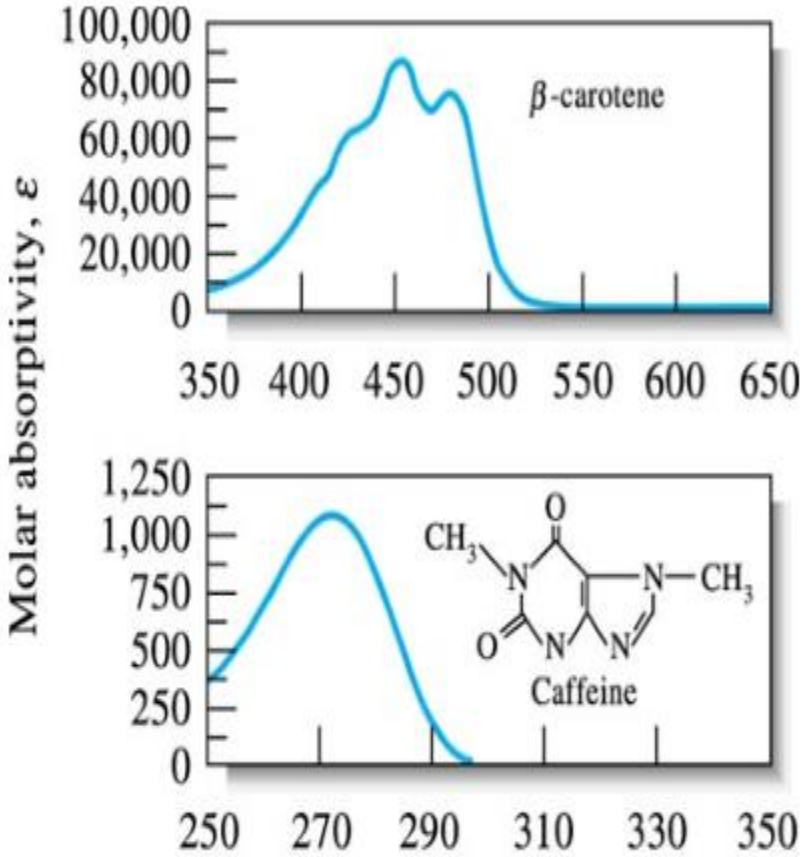
3) n → π* transition

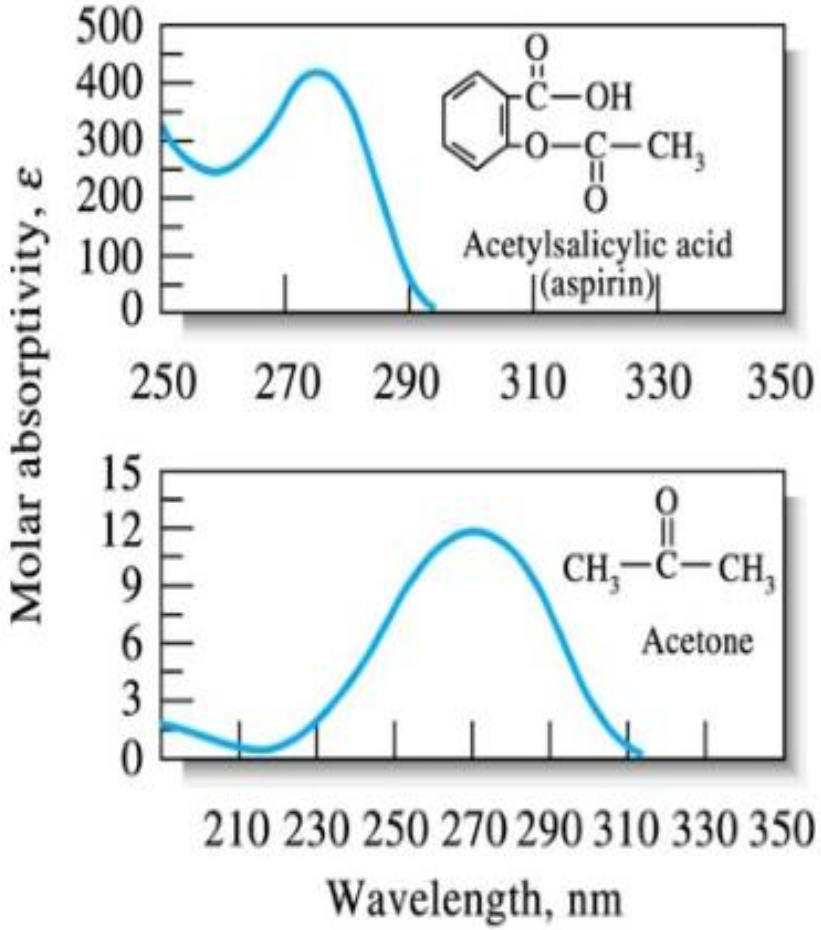
- usually observed in molecules of an unsaturated group (halogen).
- Example: carbonyl band (C=O)→ 270-295 nm

4) π → π* transition

- compounds possessing an isolated ethylenic double bond (C=C) →strong adsorption band ~ 170 nm.
- Example: Ethylene: λ_{max} = 165 nm

Examples: Typical Absorption Spectra





Absorption characteristic of some common Chromophores

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- Molecules containing unsaturated organic functional groups and capable of absorbing UV/visible radiation.

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	$n \rightarrow \pi^*$

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Molecular Spectroscopy

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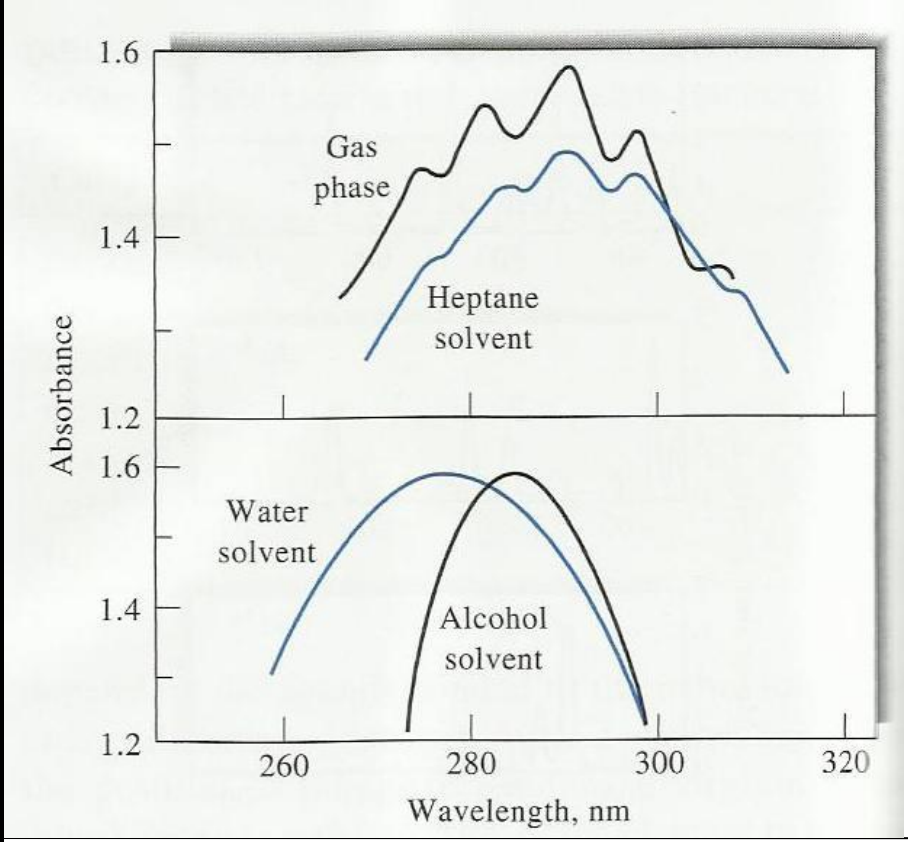
- Fourier Transform Infrared (FTIR)
- Organic UV-Vis Absorption
- Effect of Solvent
- Inorganic UV-Vis Absorption
- Qualitative Analysis
- Quantitative Analysis
- Molecular Fluorescence
- Equipment for Molecular Spectroscopy

Effect of Solvent

- The position and intensity of the absorption bands will vary with the nature of the solvent used → each solvent has its own characteristic polarity.



Effect of solvent on the absorption spectrum of acetaldehyde

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Common solvent for UV/Vis Region

TABLE 26-3

Solvents for the Ultraviolet and Visible Regions

Solvent	Lower Wavelength Limit, nm	Solvent	Lower Wavelength Limit, nm
Water	180	Carbon tetracholoride	260
Ethanol	220	Diethyl ether	210
Hexane	200	Acetone	330
Cyclohexane	200	Dioxane	320
		Cellosolve	320

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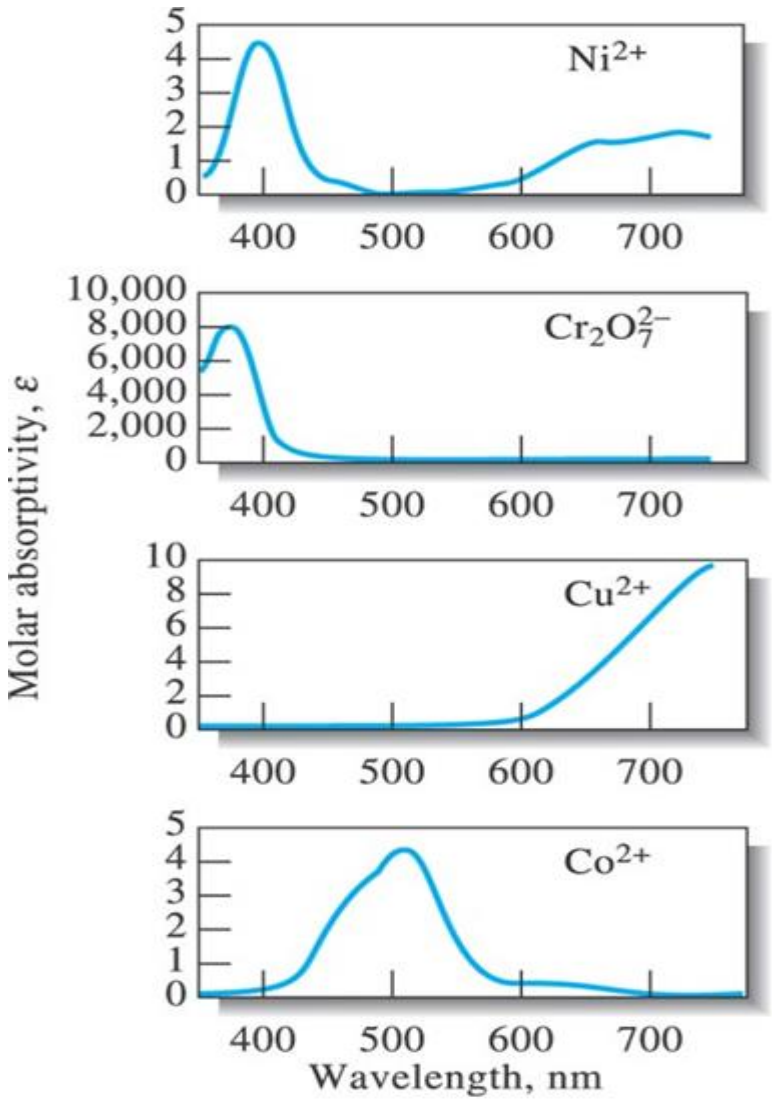
Inorganic UV/Vis Absorption: Absorption by Organic Compounds

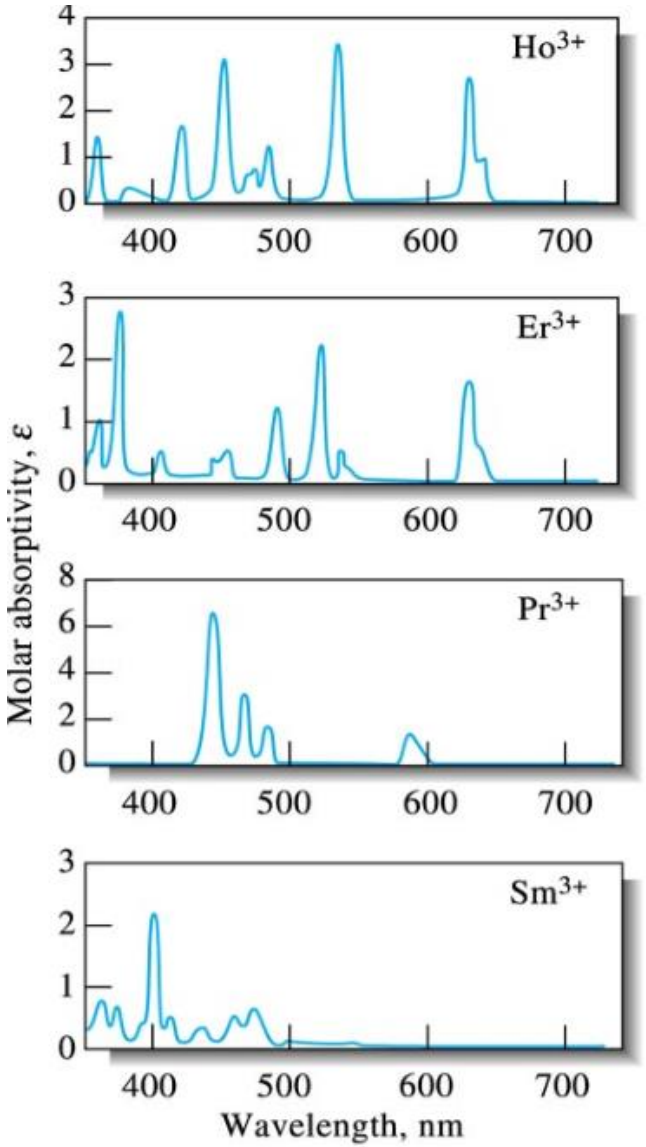
- A number of inorganic anions exhibit UV absorption bands.

- Example: nitrate, nitrite and chromate.

Examples:



Absorption spectra of aqueous solutions of transition metal ions



Absorption spectra of aqueous solutions of rare earth ions

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Molecular Spectroscopy

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Qualitative Applications of UV/Vis Absorption Spectroscopy

- Detecting chromophoric groups.
- The appearance of one or more peaks in the region from 200-400 nm is clear indication of the presence of unsaturated groups or of atoms such as sulfur or halogens.
- Spectral position → indication of the presence or absence of certain structural features or functional groups in a molecules.
- Identity of the absorbing groups → can be determined by comparing the spectrum of an analyte with those of simple molecules containing various chromophoric groups.

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Quantitative Analysis

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- UV/Vis absorption spectroscopy → most useful tools available for quantitative analysis.

- Important characteristic of spectrophotometric method:
  1. wide applicability to both organic & inorganic systems.
  2. typical detection limits to 10^{-4} to 10^{-5} M.
  3. good accuracy (1-3%)
  4. ease and convenience of data acquisition.

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Method of Analysis

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1) External Standard (calibration curve)

2) Standard Addition

   i) Single-point addition

   ii) Multiple additions

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External Standard (Calibration Curve)

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- A standard solutions of the analyte is used to construct a calibration curve of absorbance versus concentration.

- The slope of the calibration curve → product of absorptivity and pathlength.

- External standard → determining the proportionality factor between absorbance and concentration under the same conditions and with the same instrument as is used for the samples.

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Example : The method of external standards

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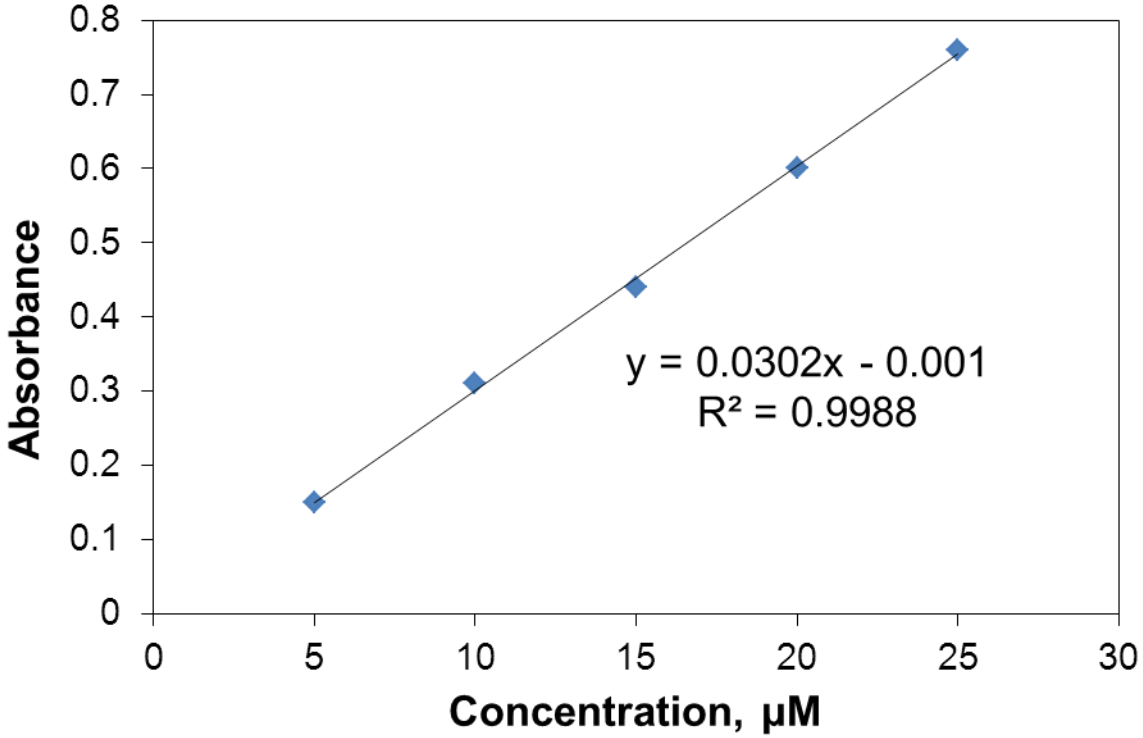
Concentration, μM	Measured Absorbance
5.00	0.150
10.00	0.310
15.00	0.440
20.00	0.600
25.00	0.760
unknown	0.421



Determine the concentration of unknown:

y = 0.0302 x -0.001

0.421 = 0.0302 x -0.001

X = 13.97 µM

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Standard Addition Method

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- For those samples having difficulties in the production of standards with an overall composition.

i) Single-point addition method:

- a known amount of analyte is introduced into a second aliquot of the sample.
- the difference in absorbance is used to calculate the analyte concentration of the sample.

ii) Multiple additions method:

-multiple additions can be made to several aliquots of the sample and multiple standard addition calibration curve is obtained.

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Example: Single-point Addition Method

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The single-point standard solution addition method was used in the determination of phosphate by the molybdenum blue method.

A 2.00 mL urine sample was treated with molybdenum blue reagents to produce a species absorbing at 820.0 nm, after which the sample was diluted to 100.00 mL.

A 25.00 mL aliquot gave an absorbance of 0.428 (solution 1). Addition of 1.00 mL of a solution containing 0.0500 mg of phosphate to a second 25.0 mL aliquot gave an absorbance of 0.517 (solution 2).

Use these data to calculate the number of mg of phosphate per mL of the sample.

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Solution

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The absorbance of the first solution is given by (Beer's Law):

$$A_1 = \varepsilon b c_x$$

where c_x is the unknown concentration of phosphate in the first solution. The absorbance if the second solution is given by:

$$A_2 = \varepsilon b V_x c_x / V_t + \varepsilon b V_s c_s / V_t$$

$m_1v_1 = m_2v_2$

V_x =volume of the solution of unknown phosphate concentration (25.00 mL).

V_s = volume of the standard solution of phosphate added (1.00 mL).

V_t = total volume after the addition (26.00 mL).

c_s = the concentration of the standard solution (0.0500 mg / 1.00 mL = 0.0500 mg/mL).

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If we solve the first equation for εb, substitute the result into the second equation, and solve for c_x, we obtain:

$$c_x = \frac{A_1 c_s V_s}{A_2 V_t - A_1 V_x}$$

$$c_x = \frac{0.428 \times 0.05\ mg\ mL^{-1} \times 1.00\ mL}{0.517 \times 26.00\ mL - 0.428 \times 25.00 mL} = 0.0078\ mg\ mL^{-1}$$

This result is the concentration of the diluted sample. To obtain the concentration of the original urine sample, we need to multiply by 100 mL/2 mL, Thus,

Concentration of phosphate = 0.0078 mg mL^{-1} x 100 mL/ 2 mL
= 0.390 mg mL^{-1}

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Example: Multiple Standard Addition Method

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A multiple standard addition method was used for determining Fe^{3+} in a natural water sample.

10.0 mL aliquots of the sample were pipetted into 50.00 mL volumetric flasks, respectively.

Exactly 0.00, 5.00, 10.00, 15.00 and 20.00 mL of a standard solution containing 11.1 ppm of Fe^{3+} were added to each followed by an excess of thiocynate ion to give the red complex $Fe(SCN)^{2+}$.

After dilution to volume, absorbances of the five solutions were measured in a 0.982 cm cell at 480.0 nm and found to be 0.240,0.437,0.621,0.809 and 1.009, respectively.

What is the concentration of Fe^{3+} in the water sample?

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Solution

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For several identical aliquots of a standard solution having a known concentration c_s, the absorbance after each addition, A_s, is given by:

$$A_s = \frac{\varepsilon b V_s c_s}{V_t} + \frac{\varepsilon b V_x c_x}{V_t} = kV_s c_s + kV_x c_x$$

V_t = total volume of each flask

V_s = variable volume of standard added

c_x = unknown concentration

k = constant equal to $\varepsilon b/V_t$

V_x = volume of the solution of unknown sample

A plot of A_s as a function of V_s yield a straight line of the form:

$$A_s = mV_s + b$$

where the slope m and intercept b are given by:

$$m = kc_s \text{ and } b = kV_x c_x$$

By combining these equations for the slope and intercept, the concentration of the unknown can be found as:

$$c_x = \frac{bc_s}{mV_x}$$

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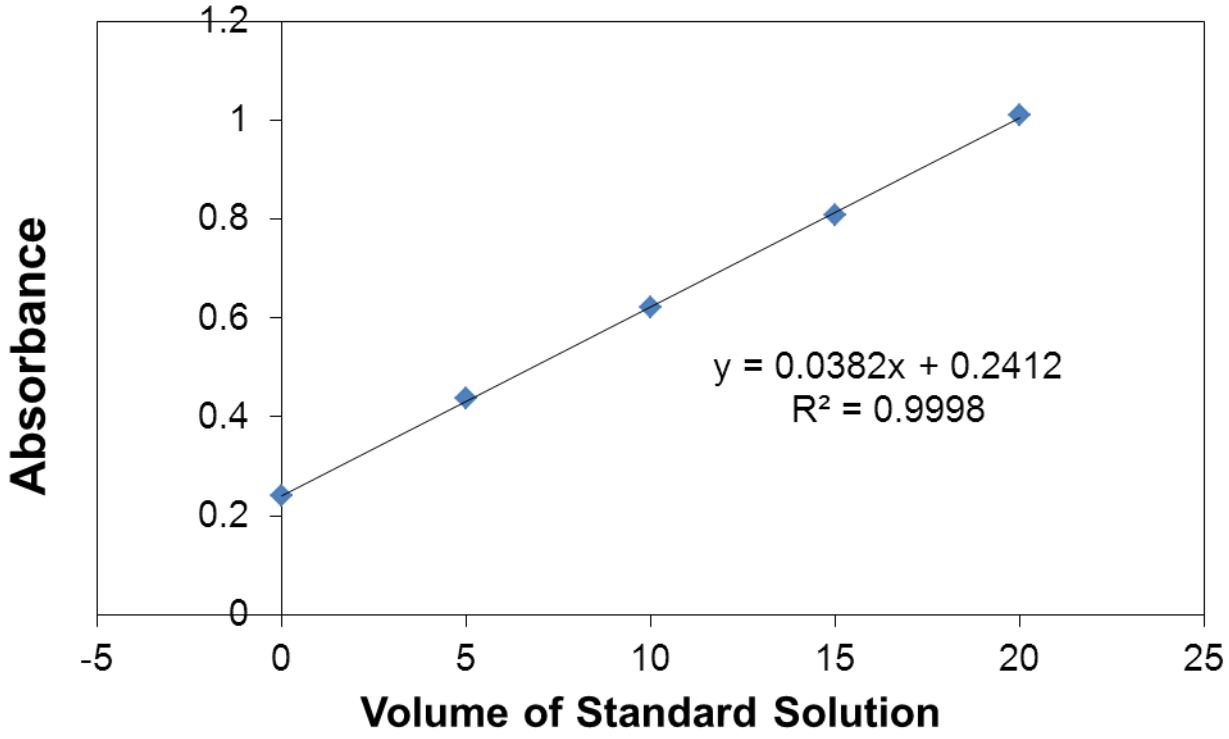
Tabulated the data

Volume of standard solution, mL (V_s)	Absorbance (A_s)
0	0.240
5	0.437
10	0.621
15	0.809
20	1.009



$$c_x = \frac{bc_s}{mV_x} = \frac{0.2412 \times 11.1\ ppm}{0.0382\ mL^{-1} \times 10\ mL} = 7.01\ ppm$$

- Absorbance vs volume →straight-line→ obeyed Beer's Law

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Exercise

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Q1:

A 25.0 mL aliquot of an aqueous quinine solution was diluted to 50.0 mL. The first 25.0 mL aliquot after the dilution had an absorbance of 0.656 at 348 nm when measured in a 2.50-cm cell. A second 25.0 mL aliquot was mixed with 10.0 mL of a solution containing 25.7 ppm of quinine. This solution had an absorbance of 0.976 (2.50 cm cell). Calculate the concentration of quinine in parts per million in the sample.

Q2:

A serum sample is analyzed for potassium by emission spectrometry using method of standard additions. Two 0.500 mL aliquots are diluted to 5.00 mL. To one portion is asses 10.0 uL of 0.0500 M KCl solution. The net emission signals in arbitrary units are 32.1 and 58.6, respectively. What is the concentration of potassium in the serum?

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Molecular Spectroscopy

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- Fourier Transform Infrared (FTIR)
- Organic UV-Vis Absorption
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- Inorganic UV-Vis Absorption
- Qualitative Analysis
- Quantitative Analysis
- Molecular Fluorescence
- Equipment for Molecular Spectroscopy

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Molecular Fluorescence Spectroscopy

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- Fluorescence → process in which atoms or molecules are excited by absorption of electromagnetic radiation. Then the excited species relax to the ground state, giving up their excess energy as photons.

- Measured by: Exciting the sample at the absorption wavelength (excitation wavelength), and measuring the emission at longer wavelength → fluorescence wavelength.

- Less widely applicable because of the relatively limited number of chemical systems that show appreciable fluorescence.

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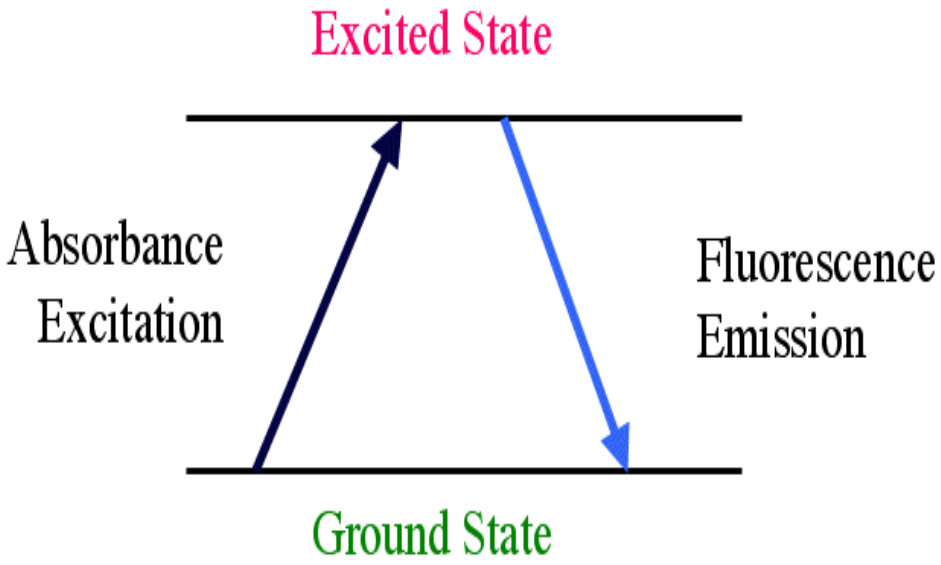
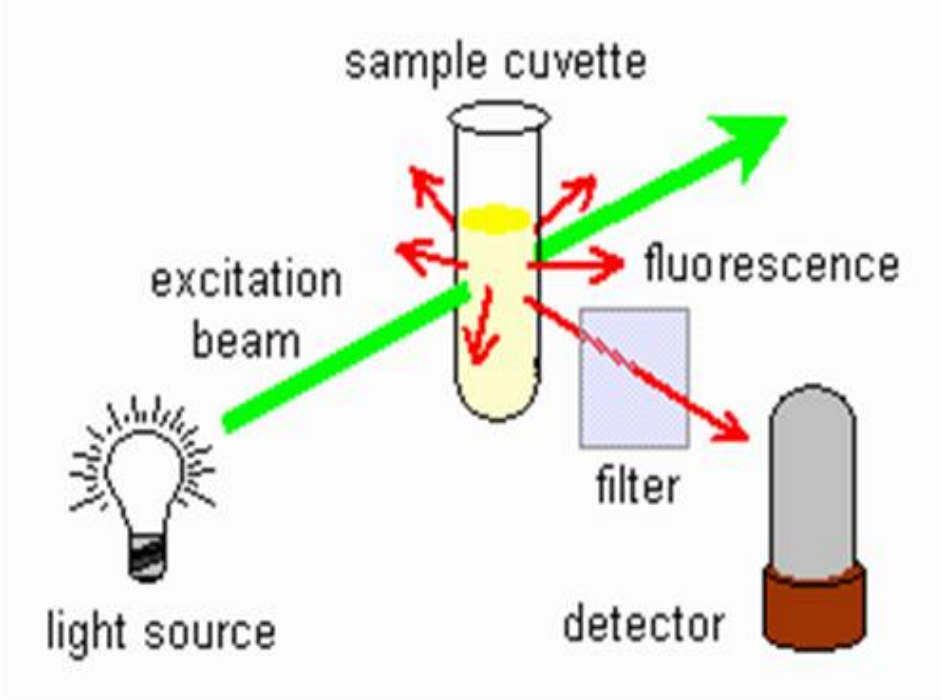
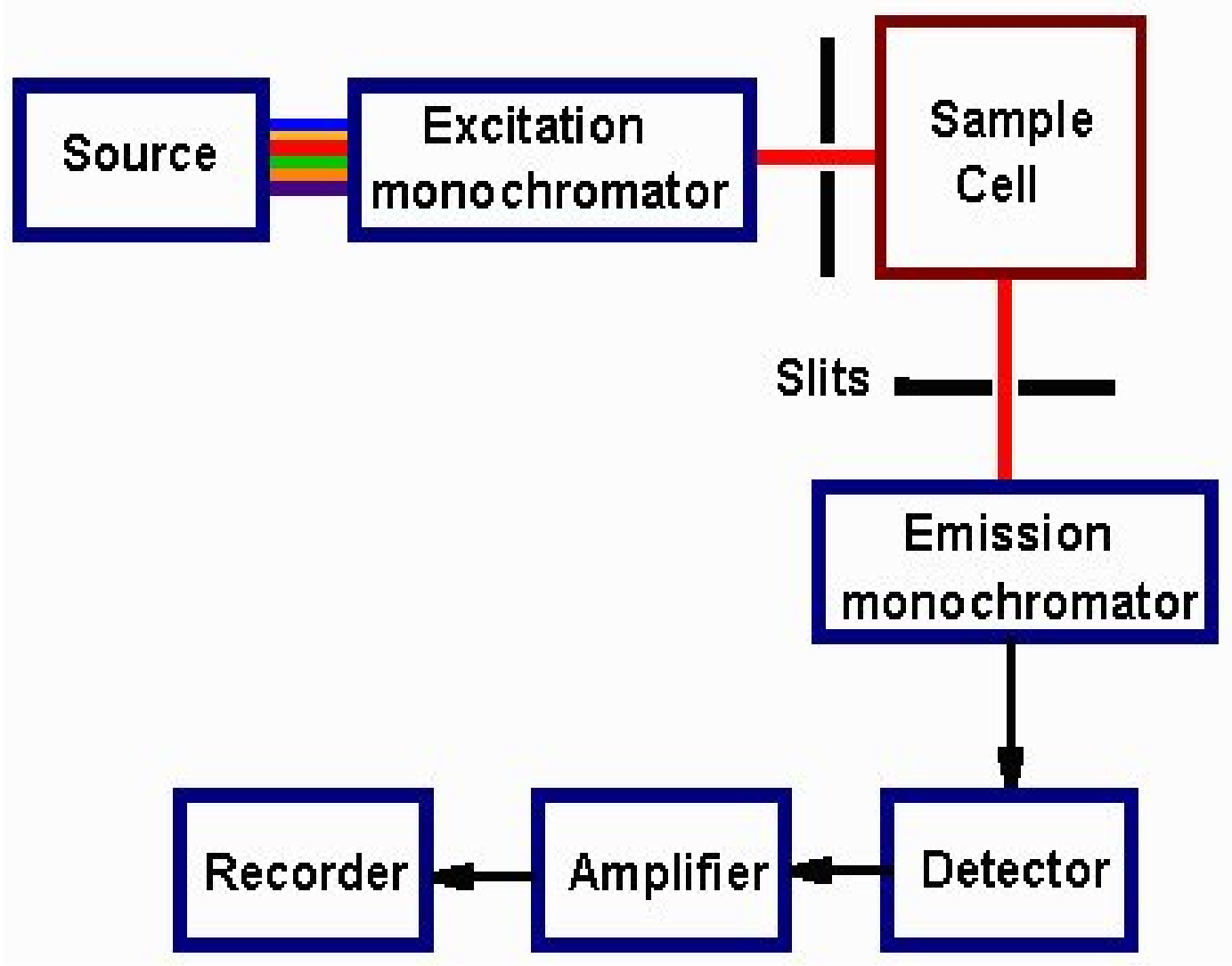






Block Diagram of Molecular Fluorescence Spectroscopy

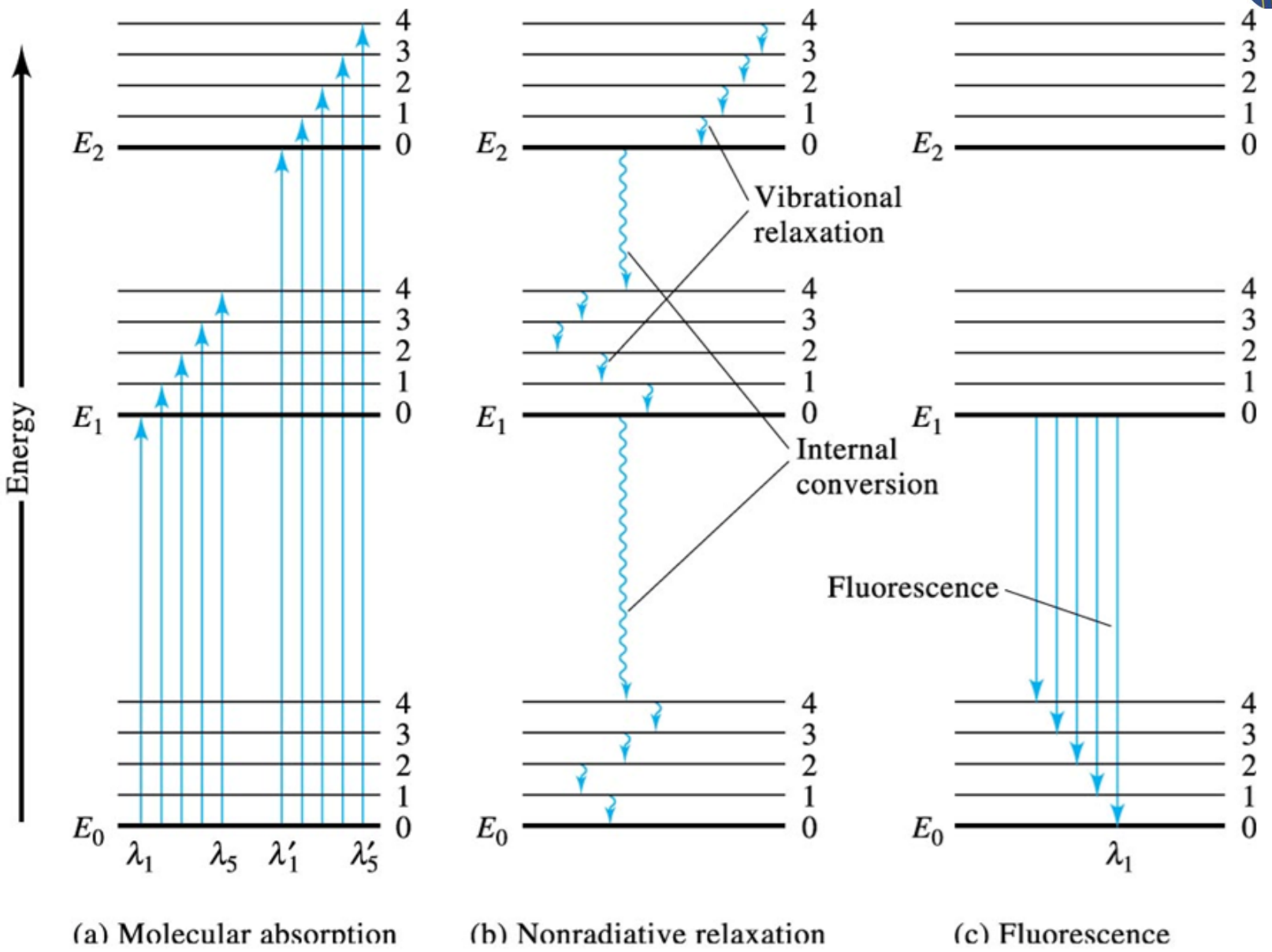
Relaxation Processes

- Once the molecule is excited to higher energy level, several processes can occur that cause the molecule to lost its excess energy .

- Two of the most important processes : i) Nonradiative Relaxation and ii) Fluorescence Emission.

- **Nonradiative Relaxation** → i) vibration relaxation and ii) internal conversion.

Energy Level Diagram: Relaxation Process



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Nonradiative Relaxation

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- Vibration Relaxation:

  - involves transfer of the excess energy of a vibrationally excited species
  - take place in less than 10^{-15} s and leaves the molecules in the lowest vibrational state of an electronic excited state.

- Internal Conversion:

  - involves transfer of the excess energy of a species in the lowest vibrational level of an excited electronic state

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Fluorescence Emission

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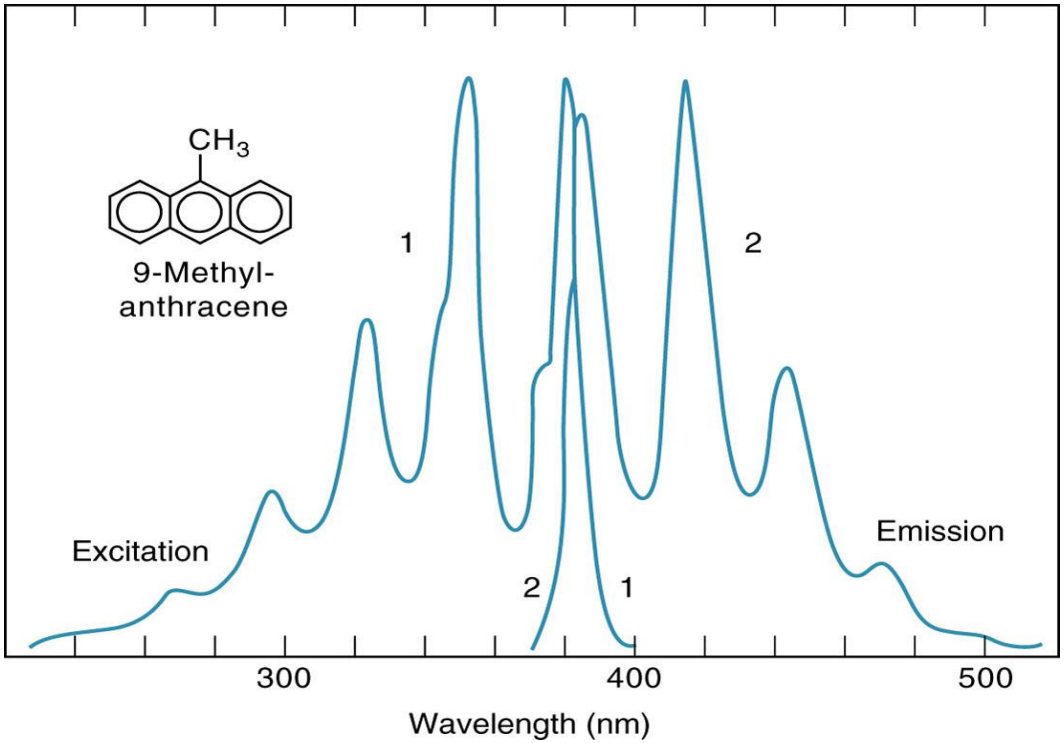
- Usually observed from the lowest lying excited electronic state E_1 to the ground state E_0 → because of the internal conversion and vibration relaxation processes are very rapid compared with fluorescence.

- Fluorescence line originate in the lowest vibrational state of E_1 → all the other line in the band are of lower energy, or longer wavelength.

- Therefore, molecular fluorescence bands →longer in wavelength, lower in frequency, and thus, lower energy than the band of absorbed radiation responsible for their excitation.

Relationship between Excitation Spectra and Fluorescence Spectra



Fluorescence spectra for 1ppm anthracene in alcohol:
(1) excitation spectrum and (2) emission spectrum (fluorescence)

The excitation spectrum and fluorescence spectrum are nearly mirror images:
→Because the energy differences between vibrational states is about the same for both ground and excited states.

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Fluorescent Species

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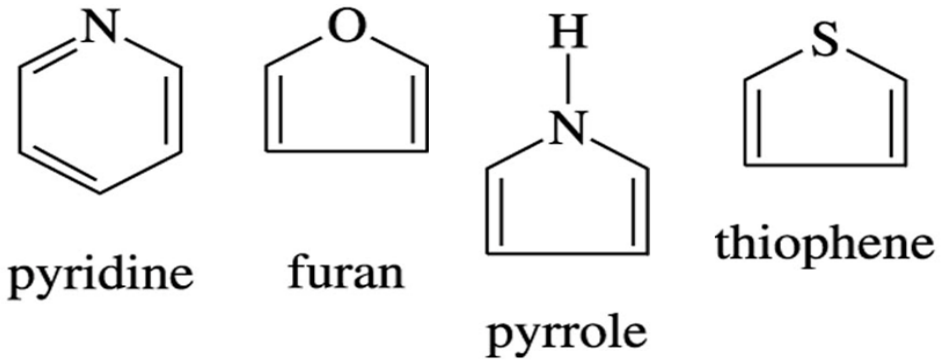
- All absorbing molecules have potential to fluoresce, but most compounds do not → their structure provides relaxation to occur at a greater rate than fluorescence.
- The Quantum Yield → the ratio of number of molecules that fluoresce to the total number of excited molecules. Or the ratio of photons emitted to photons absorbed.
- Highly fluorescent molecules → quantum efficiency approach unity.
- Nonfluorescent species → quantum efficiency approach zero.

Fluorescence and Structure



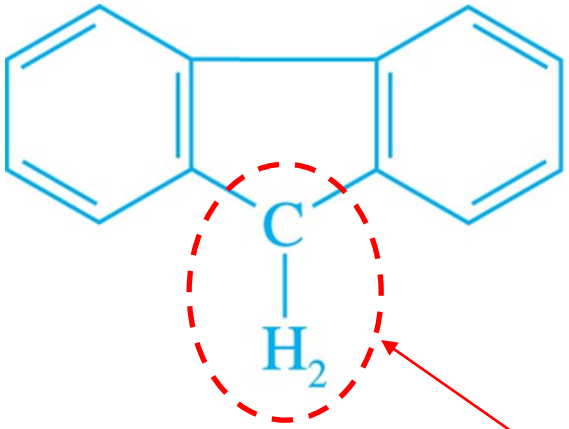
Typical aromatic molecules that do not fluoresce

Typical aromatic molecules that fluoresce

- The most intense and the most useful fluorescence is found in compounds containing aromatic functional groups.

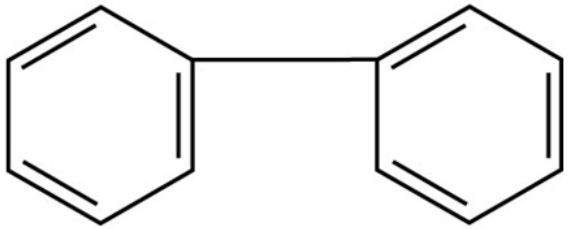
The Effect of Structural Rigidity

- Fluorescence is particular favored in rigid molecules.



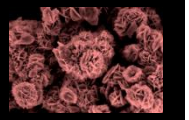
fluorene
Φ : 1

Two benzene rings in biphenyl can rotate to one another



biphenyl
Φ : 0.2

- Increase the rigidity of the fluorene
- Rigidity lower the rate of nonradiative relaxation





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Compound	Formula	Wavelength of Fluorescence, nm	Relative Intensity of Fluorescence
Benzene	C_6H_6	270–310	10
Toluene	$C_6H_5CH_3$	270–320	17
Propylbenzene	$C_6H_5C_3H_7$	270–320	17
Fluorobenzene	C_6H_5F	270–320	10
Chlorobenzene	C_6H_5Cl	275–345	7
Bromobenzene	C_6H_5Br	290–380	5
Iodobenzene	C_6H_5I	—	0
Phenol	C_6H_5OH	285–365	18
Phenolate ion	$C_6H_5O^-$	310–400	10
Anisole	$C_6H_5OCH_3$	285–345	20
Aniline	$C_6H_5NH_2$	310–405	20
Anilinium ion	$C_6H_5NH_3^+$	—	0
Benzoic acid	C_6H_5COOH	310–390	3
Benzonitrile	C_6H_5CN	280–360	20
Nitrobenzene	$C_6H_5NO_2$	—	0

Temperature and Solvent Effect

- The quantum efficiency decreases with increasing temperature.
- Increase the frequency of collision at elevated temperatures increases the probability of collisional relaxation.
- A decrease in solvent viscosity → decrease the quantum efficiency.

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Topics under Molecular Spectroscopy

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- Fourier Transform Infrared (FTIR)
- Organic UV-Vis Absorption
- Effect of Solvent
- Inorganic UV-Vis Absorption
- Qualitative Analysis
- Quantitative Analysis
- Molecular Fluorescence
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Equipment for Molecular Spectroscopy

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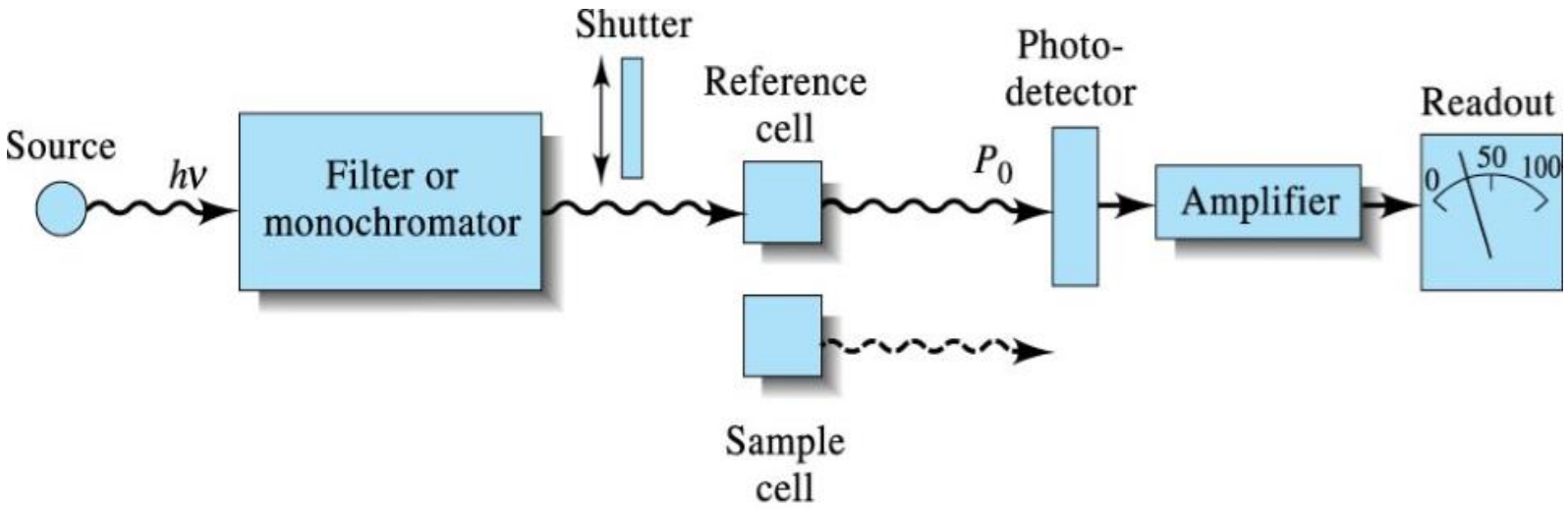
- UV/Visible spectroscopy
- Infrared spectroscopy
- Fluorescence spectroscopy

UV/Visible Instrumentation

- Molecular absorption spectrometry

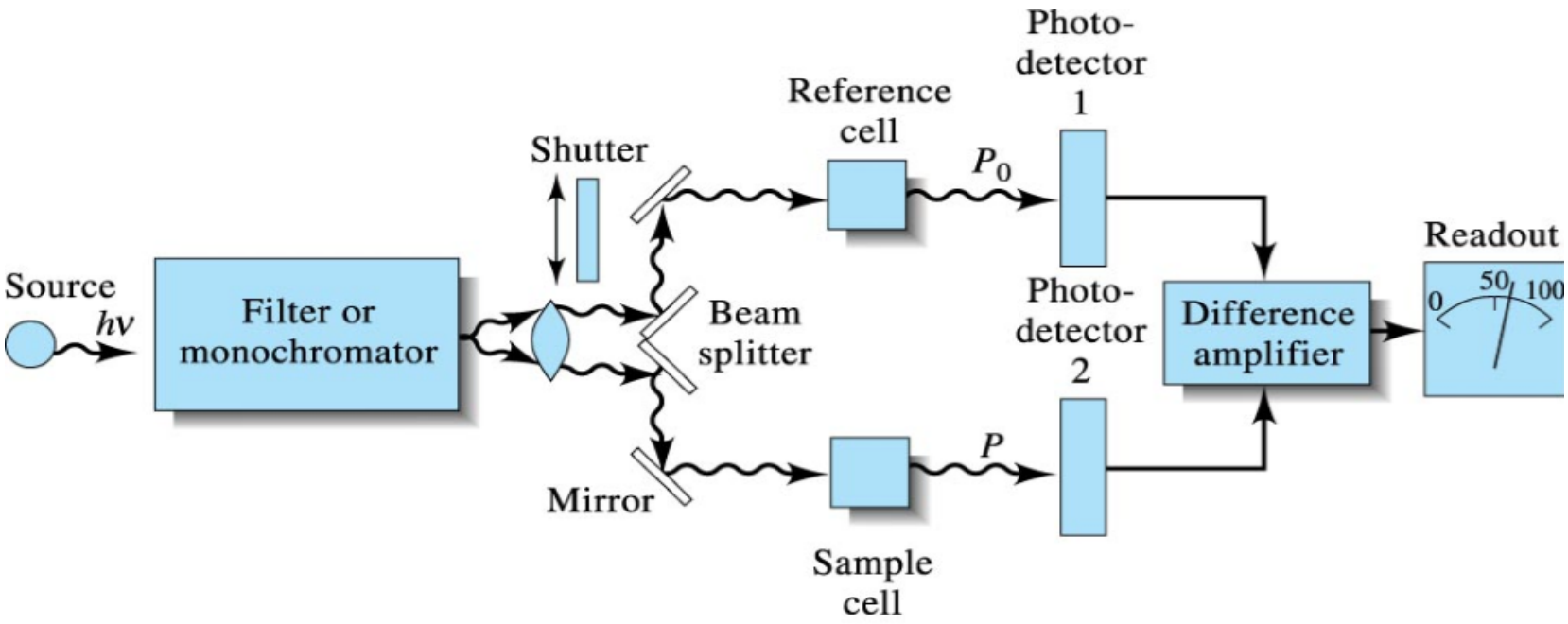
- Instrumental design for UV-visible photometers or spectrophotometer consists of four general types:
  (1) Single beam
  (2) Double beam
  (3) Double beam in time
  (4) Multichannel

UV-Vis: Single Beam Instrument



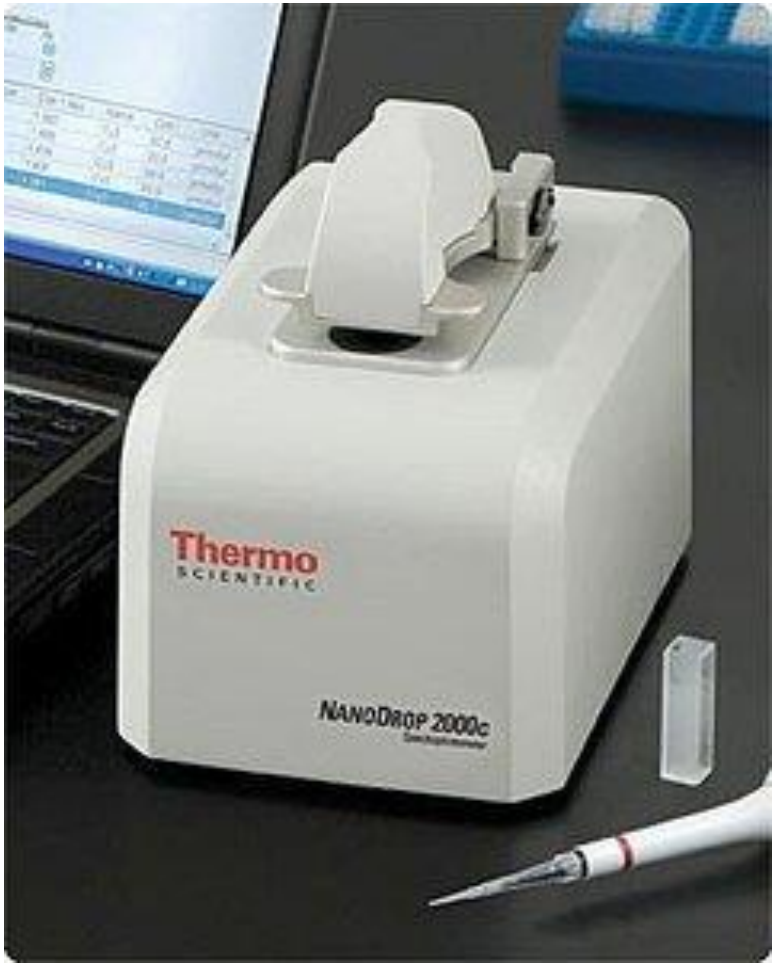
- Radiation from the filter or monochromator passes through either the reference cell or the sample cell before striking the photodetector

UV-Vis: Double Beam Instrument



- Radiation from the filter or monochromator is split into two beams that simultaneously pass through the reference and sample cells before striking two matched photodetectors.

Single beam UV/Vis Spectrophotometer



Double beam UV/Vis Spectrophotometer

Infrared (IR) Instrumentation

- Molecular absorption spectroscopy

- Instrumental design for IR spectrometer can be divided into two categories:

  (1) Fourier Transform spectrometers

  i. single beam

  ii. double beam

  (2) Dispersive spectrometer

  - generally double beam

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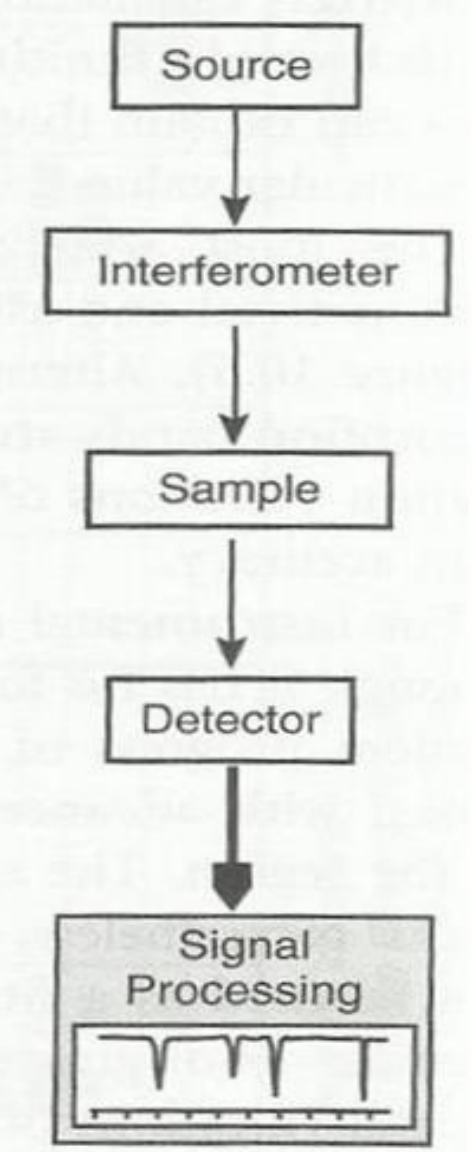

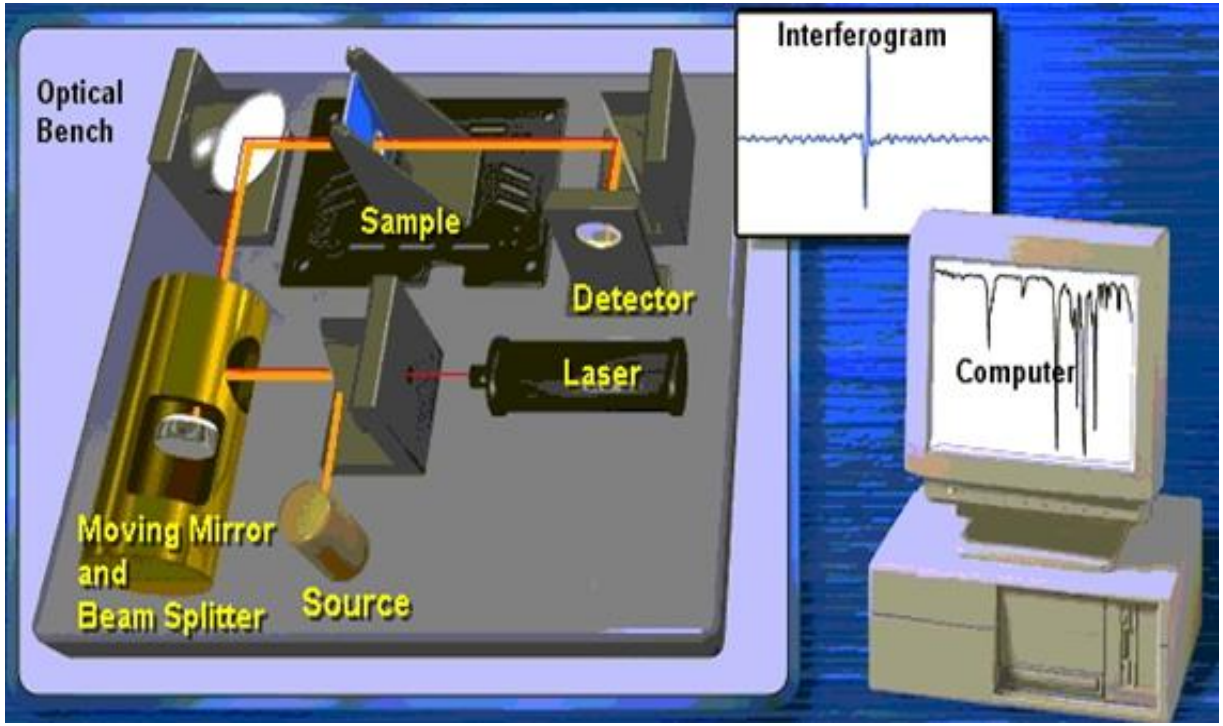

Block diagram of FT-IR spectrometer

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Fourier Transform Infrared (FTIR)

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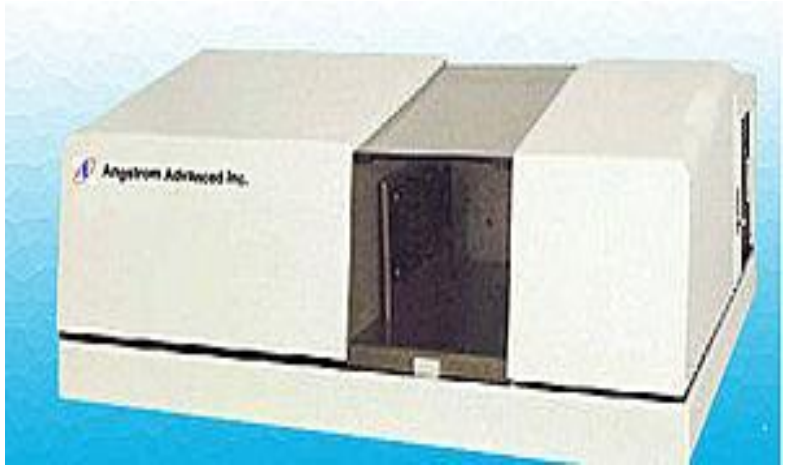
- FTIR spectrometer can be single beam or double beam instrument.

- General operation of single beam FTIR:
  i. Obtained a reference interferogram by scanning reference.
  ii. Sample is inserted in the radiation path and the process repeated.
  iii. The ratio of sample and reference spectral data is then computed to give transmittance at various frequencies.
  iv. From this ratio, the absorbance is calculated as a function of wavenumber.

FTIR Spectrometer

Single Beam



Double Beam

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Fluorescence Instrumentation

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- Molecular fluorescence spectroscopy

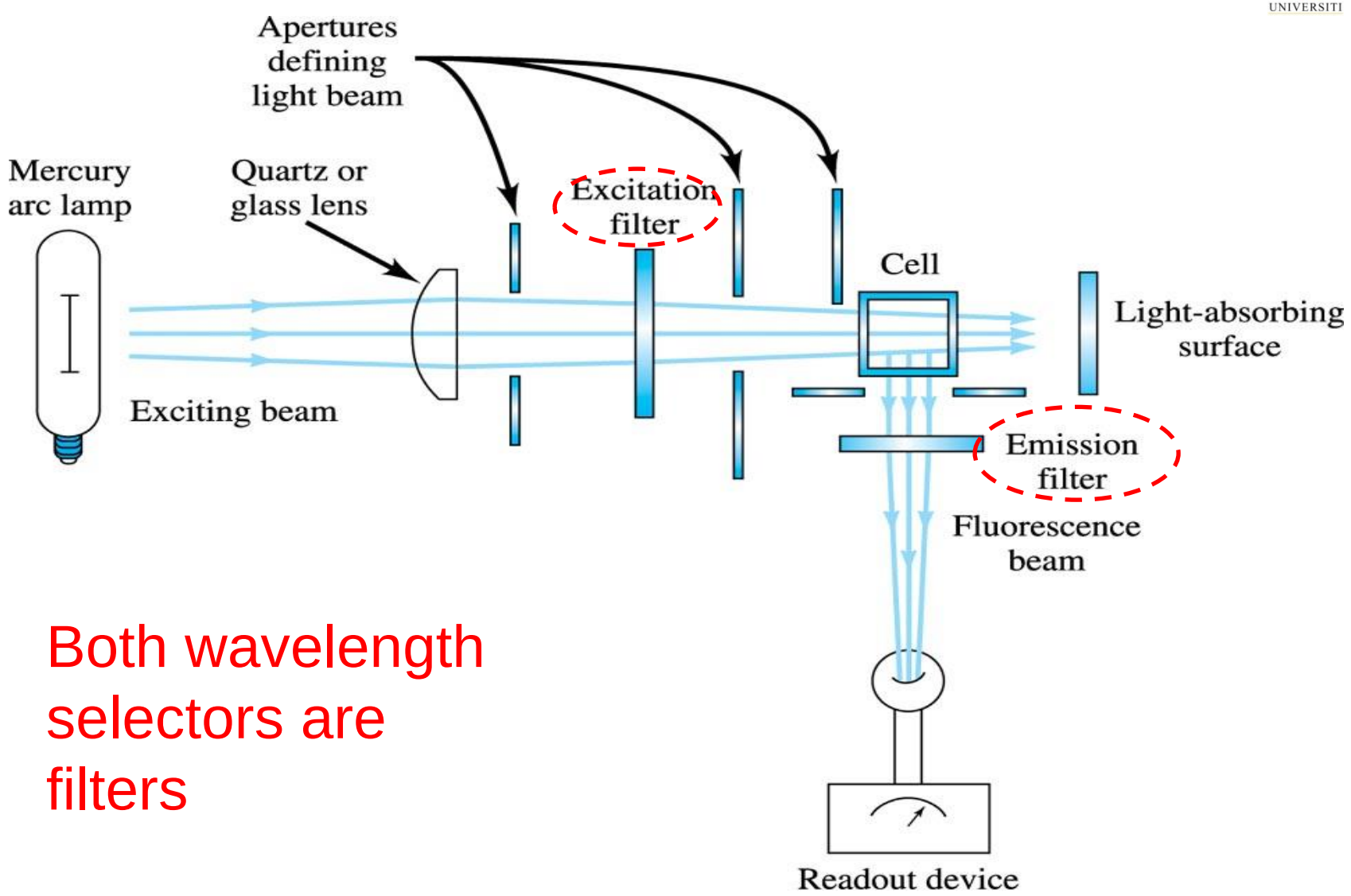
- Two types: i) Fluorometer ii) Spectrofluorometer

General operation:

i. The light emitted by primary source initially passes through the excitation monochromator which allows a narrow band of wavelengths to be selected.

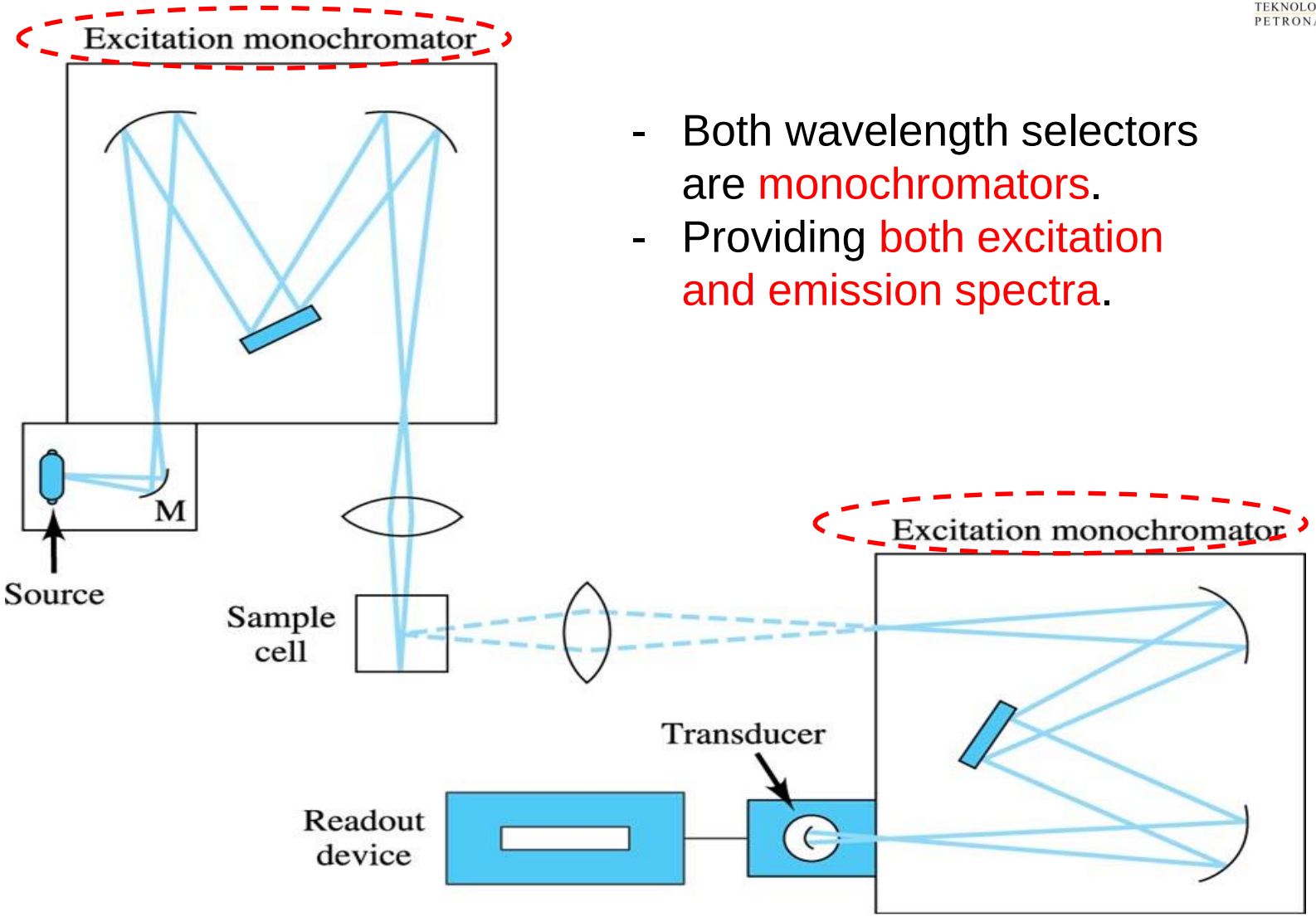
ii. A part of the fluorescence emitted is collected and passes through the emission monochromator allowing the selection of a narrow band of wavelength for measurement.

Fluorometer



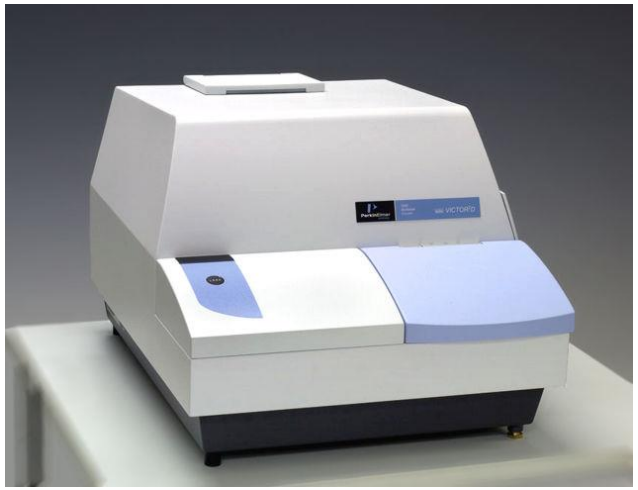
Both wavelength selectors are filters

Spectrofluorometer

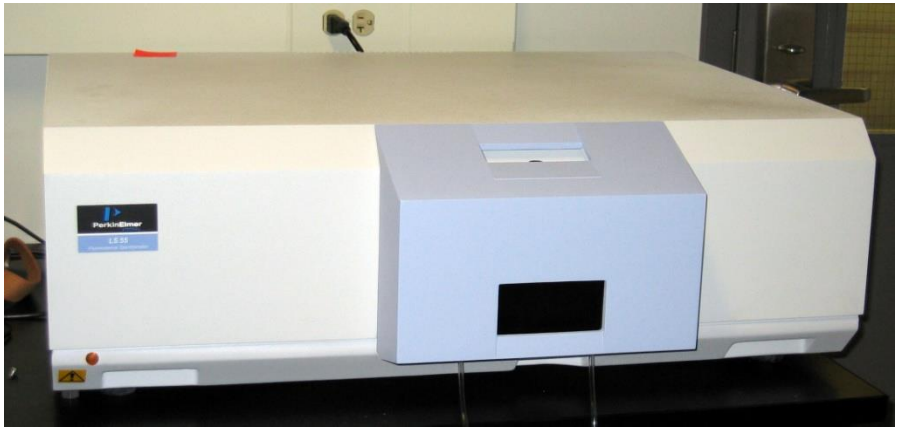


- Both wavelength selectors are monochromators.
- Providing both excitation and emission spectra.

Fluorescence Spectrophotometer (fluorometer)



Fluorescence Spectrometer (spectrofluorometer)



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