



UV / VISIBLE SPECTROSCOPY

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Spectroscopy

- It is the branch of science that deals with the study of interaction of matter with light.

OR

- It is the branch of science that deals with the study of interaction of electromagnetic radiation with matter.



Electromagnetic Radiation

Electromagnetic Radiation

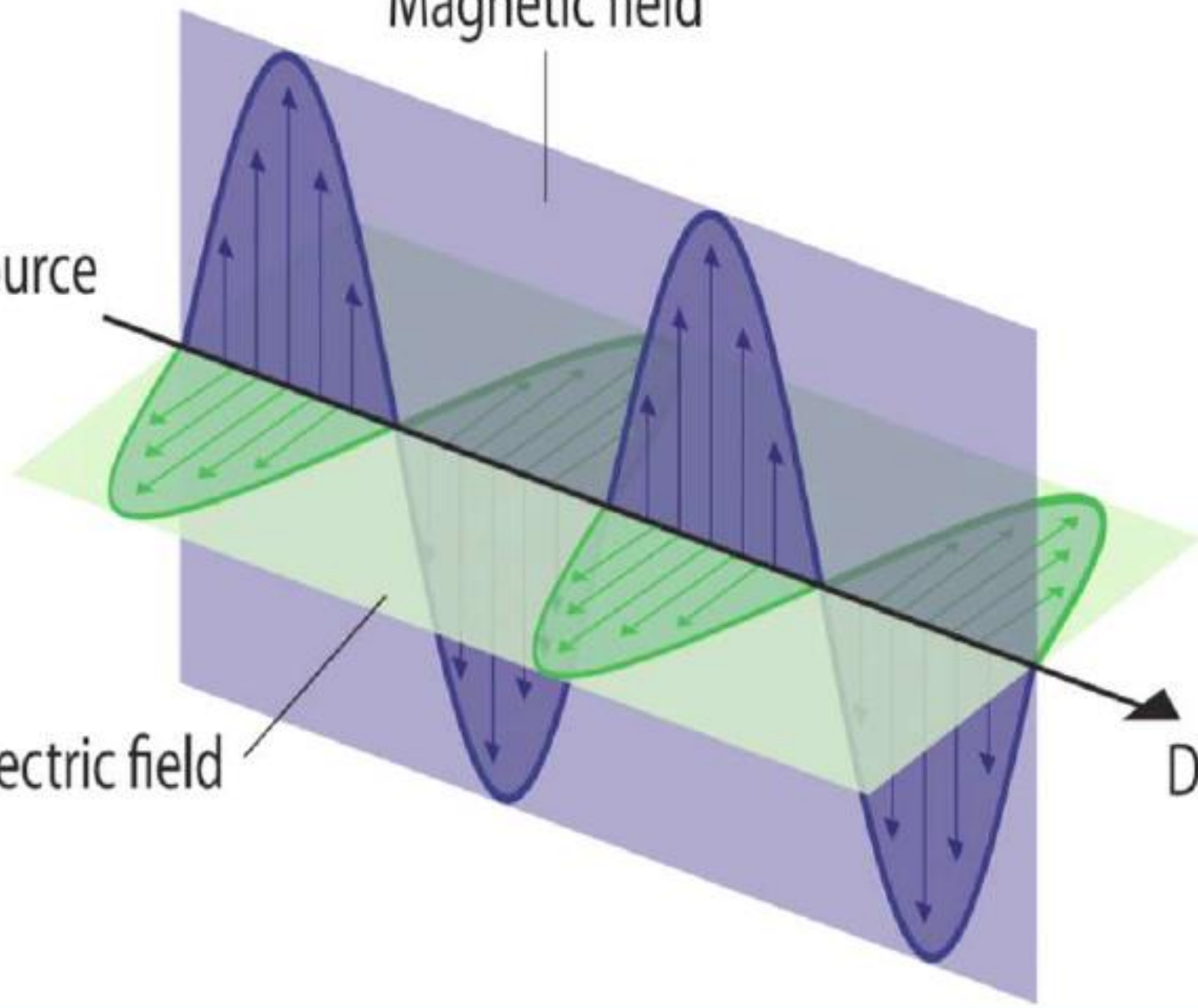
- Electromagnetic radiation consist of discrete packets of energy which are called as photons.
- A photon consists of an oscillating electric field (E) & an oscillating magnetic field (M) which are perpendicular to each other.

Magnetic field

Source

Electric field

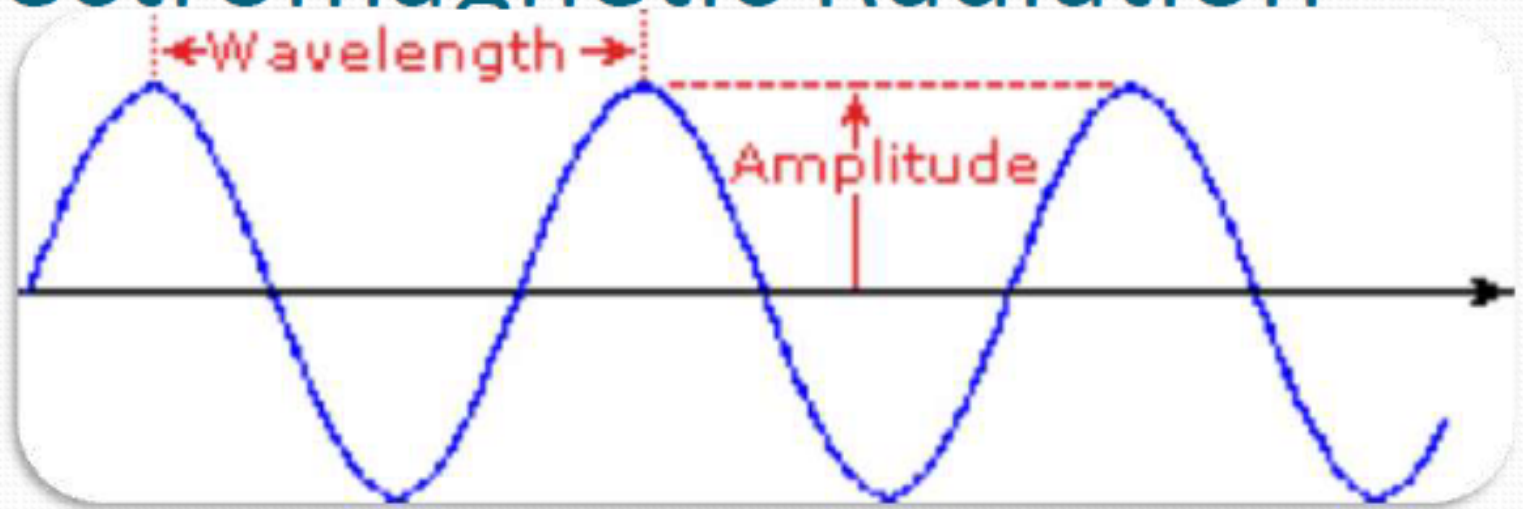
Direction



Electromagnetic Radiation

- Frequency (ν):
 - It is defined as the number of times electrical field radiation oscillates in one second.
 - The unit for frequency is Hertz (Hz).
 $1 \text{ Hz} = 1 \text{ cycle per second}$
- Wavelength (λ):
 - It is the distance between two nearest parts of the wave in the same phase i.e. distance between two nearest crest or troughs.

Electromagnetic Radiation



- The relationship between wavelength & frequency can be written as:

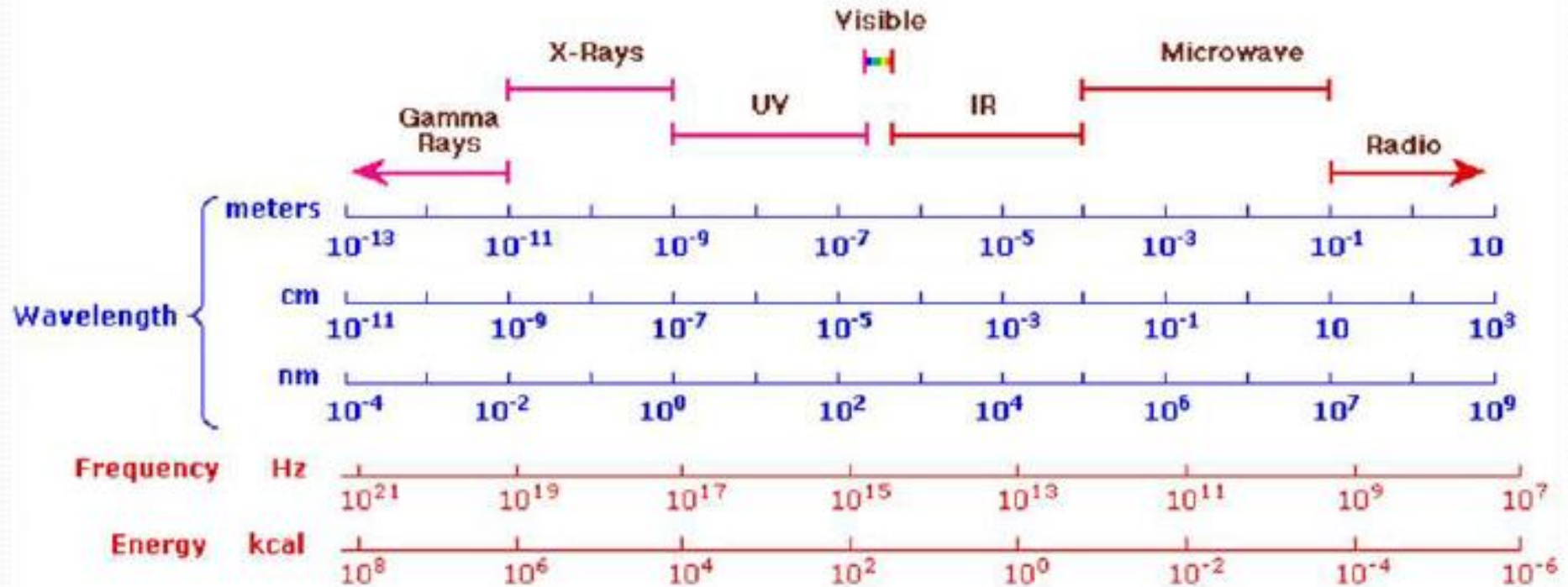
$$c = \nu \lambda$$

- As photon is subjected to energy, so

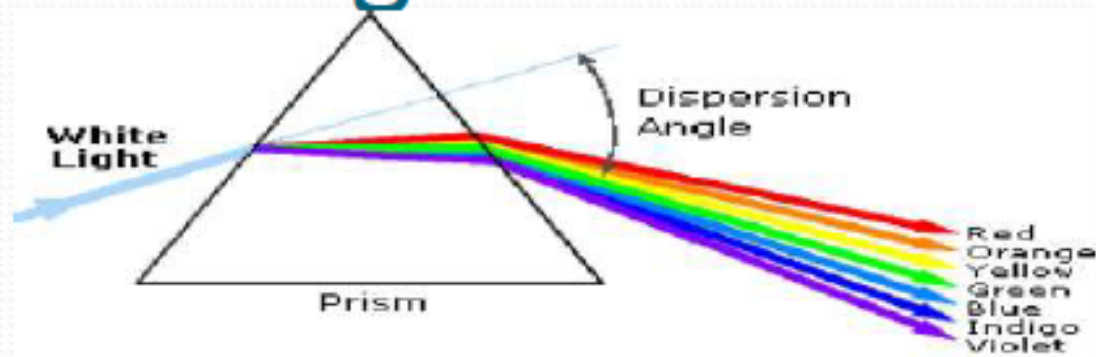
$$E = h \nu = h c / \lambda$$

Electromagnetic Radiation

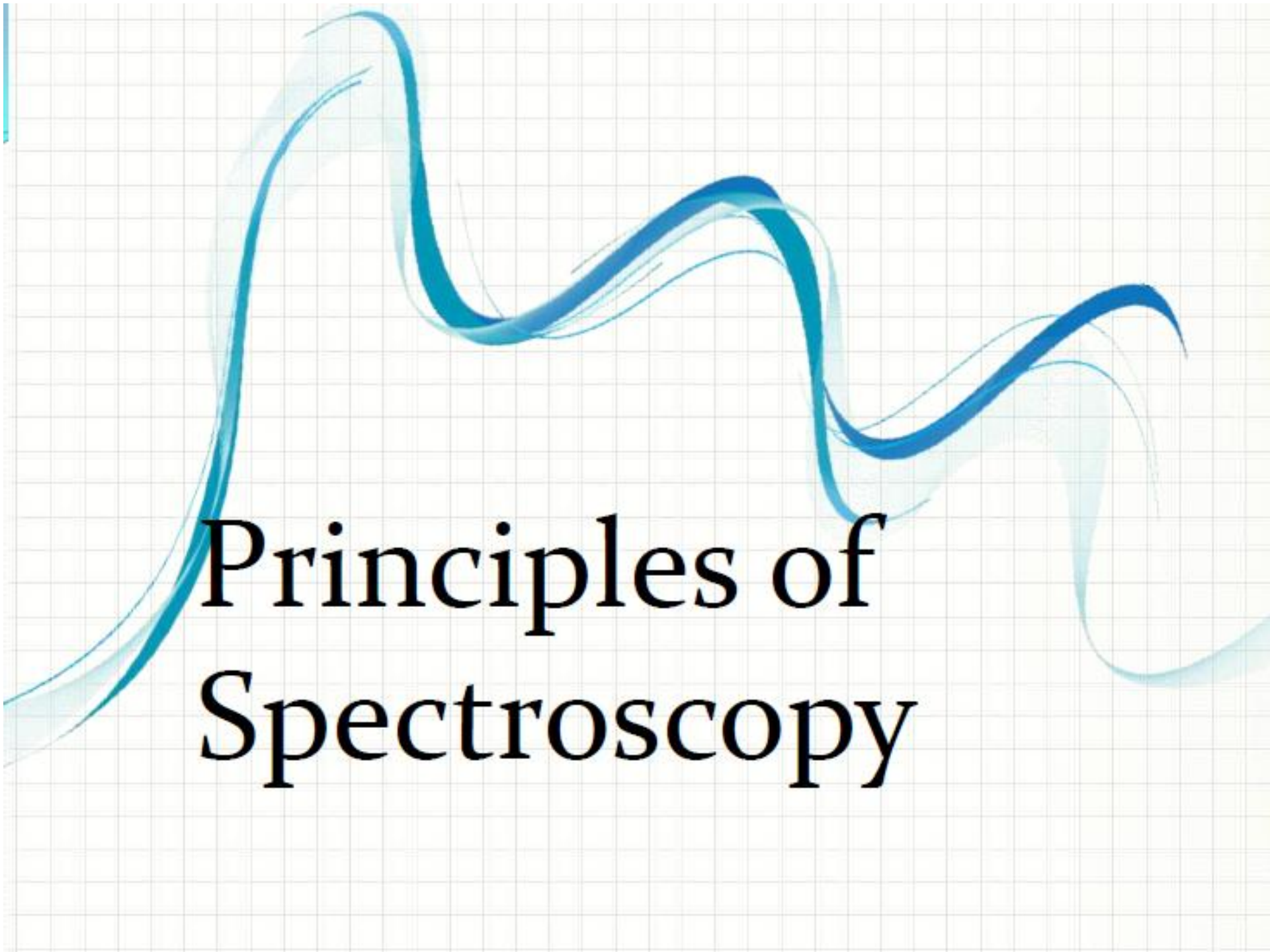
The Electromagnetic Spectrum



Electromagnetic Radiation



Violet	400 - 420 nm	Yellow	570 - 585 nm
Indigo	420 - 440 nm	Orange	585 - 620 nm
Blue	440 - 490 nm	Red	620 - 780 nm
Green	490 - 570 nm		



Principles of Spectroscopy

Principles of Spectroscopy

- The principle is based on the measurement of spectrum of a sample containing atoms / molecules.
- Spectrum is a graph of intensity of absorbed or emitted radiation by sample verses frequency (ν) or wavelength (λ).
- Spectrometer is an instrument design to measure the spectrum of a compound.

Principles of Spectroscopy

1. Absorption Spectroscopy:

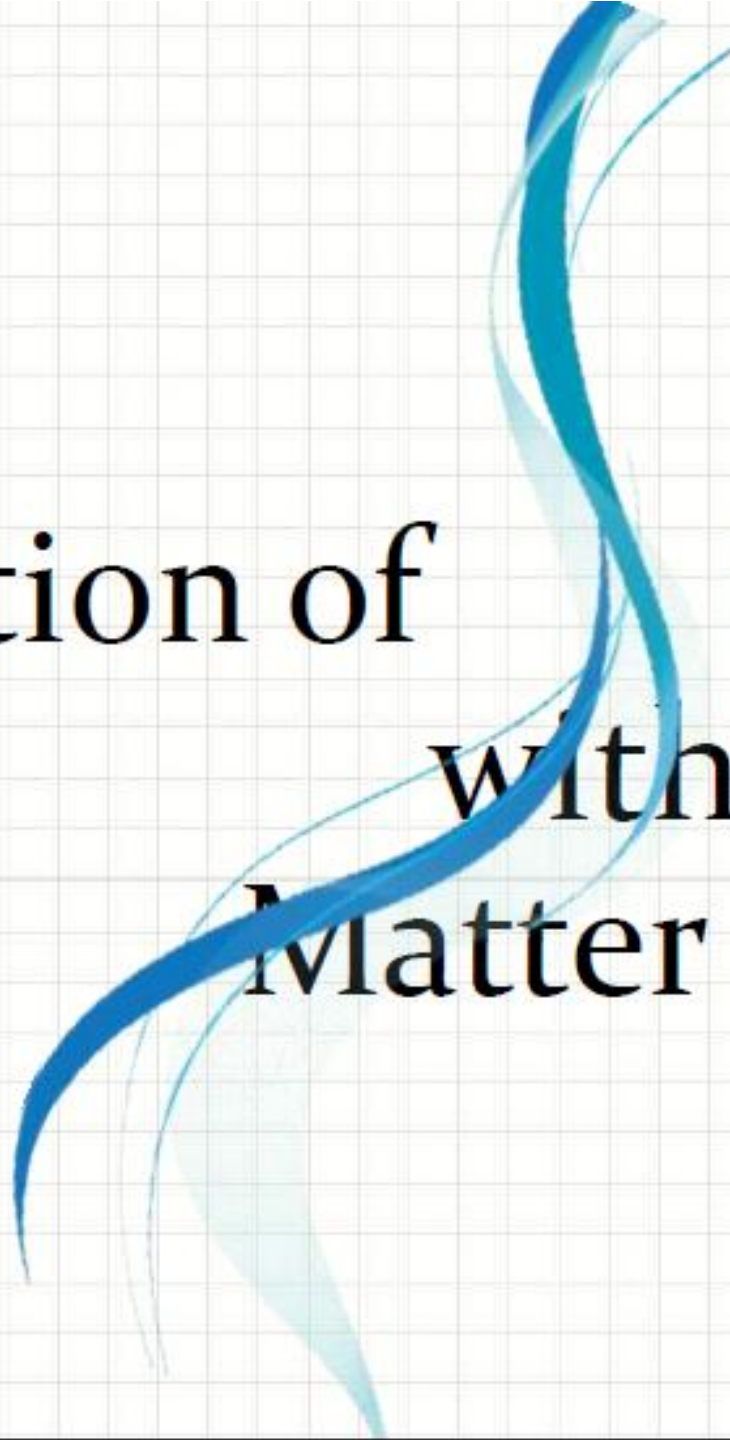
- An analytical technique which concerns with the measurement of absorption of electromagnetic radiation.
- e.g. UV (185 - 400 nm) / Visible (400 - 800 nm) Spectroscopy, IR Spectroscopy (0.76 - 15 μm)

Principles of Spectroscopy

2. Emission Spectroscopy:

- An analytical technique in which emission (of a particle or radiation) is dispersed according to some property of the emission & the amount of dispersion is measured.

- e.g. Mass Spectroscopy

A decorative graphic consisting of several overlapping, wavy blue lines that curve from the top right towards the bottom left, partially obscuring the text.

Interaction of EMR with Matter

Interaction of EMR with matter

1. Electronic Energy Levels:

- At room temperature the molecules are in the lowest energy levels E_0 .
- When the molecules absorb UV-visible light from EMR, one of the outermost bond / lone pair electron is promoted to higher energy state such as $E_1, E_2, \dots E_n$, etc is called as electronic transition and the difference is as:

$$\Delta E = h \nu = E_n - E_0 \quad \text{where } (n = 1, 2, 3, \dots \text{ etc})$$

$$\Delta E = 35 \text{ to } 71 \text{ kcal/mole}$$

Interaction of EMR with matter

2. Vibrational Energy Levels:

- These are less energy level than electronic energy levels.
- The spacing between energy levels are relatively small i.e. 0.01 to 10 kcal/mole.

e.g. when IR radiation is absorbed, molecules are excited from one vibrational level to another or it vibrates with higher amplitude.

3. Rotational Energy Levels:

- These energy levels are quantized & discrete.
- The spacing between energy levels are even smaller than vibrational energy levels.

$$\Delta E_{\text{rotational}} < \Delta E_{\text{vibrational}} < \Delta E_{\text{electronic}}$$

The possible electronic transitions are

1

• $\sigma \rightarrow \sigma^*$ transition

2

• $\pi \rightarrow \pi^*$ transition

3

• $n \rightarrow \sigma^*$ transition

4

• $n \rightarrow \pi^*$ transition

5

• $\sigma \rightarrow \pi^*$ transition

6

• $\pi \rightarrow \sigma^*$ transition

1

• $\sigma \rightarrow \sigma^*$ transition

- σ electron from orbital is excited to corresponding anti-bonding orbital σ^* .
- The energy required is large for this transition.
- e.g. Methane (CH_4) has C-H bond only and can undergo $\sigma \rightarrow \sigma^*$ transition and shows absorbance maxima at 125 nm.

2

• $\pi \rightarrow \pi^*$ transition

- π electron in a bonding orbital is excited to corresponding anti-bonding orbital π^* .
- Compounds containing multiple bonds like alkenes, alkynes, carbonyl, nitriles, aromatic compounds, etc undergo $\pi \rightarrow \pi^*$ transitions.
 - e.g. Alkenes generally absorb in the region 170 to 205 nm.

3

• $n \rightarrow \sigma^*$ transition

- Saturated compounds containing atoms with lone pair of electrons like O, N, S and halogens are capable of $n \rightarrow \sigma^*$ transition.
- These transitions usually requires less energy than $\sigma \rightarrow \sigma^*$ transitions.
- The number of organic functional groups with $n \rightarrow \sigma^*$ peaks in UV region is small (150 – 250 nm).

4

• $n \rightarrow \pi^*$ transition

- An electron from non-bonding orbital is promoted to anti-bonding π^* orbital.
- Compounds containing double bond involving hetero atoms ($C=O$, $C\equiv N$, $N=O$) undergo such transitions.
- $n \rightarrow \pi^*$ transitions require minimum energy and show absorption at longer wavelength around 300 nm.

5

- $\sigma \rightarrow \pi^*$ transition

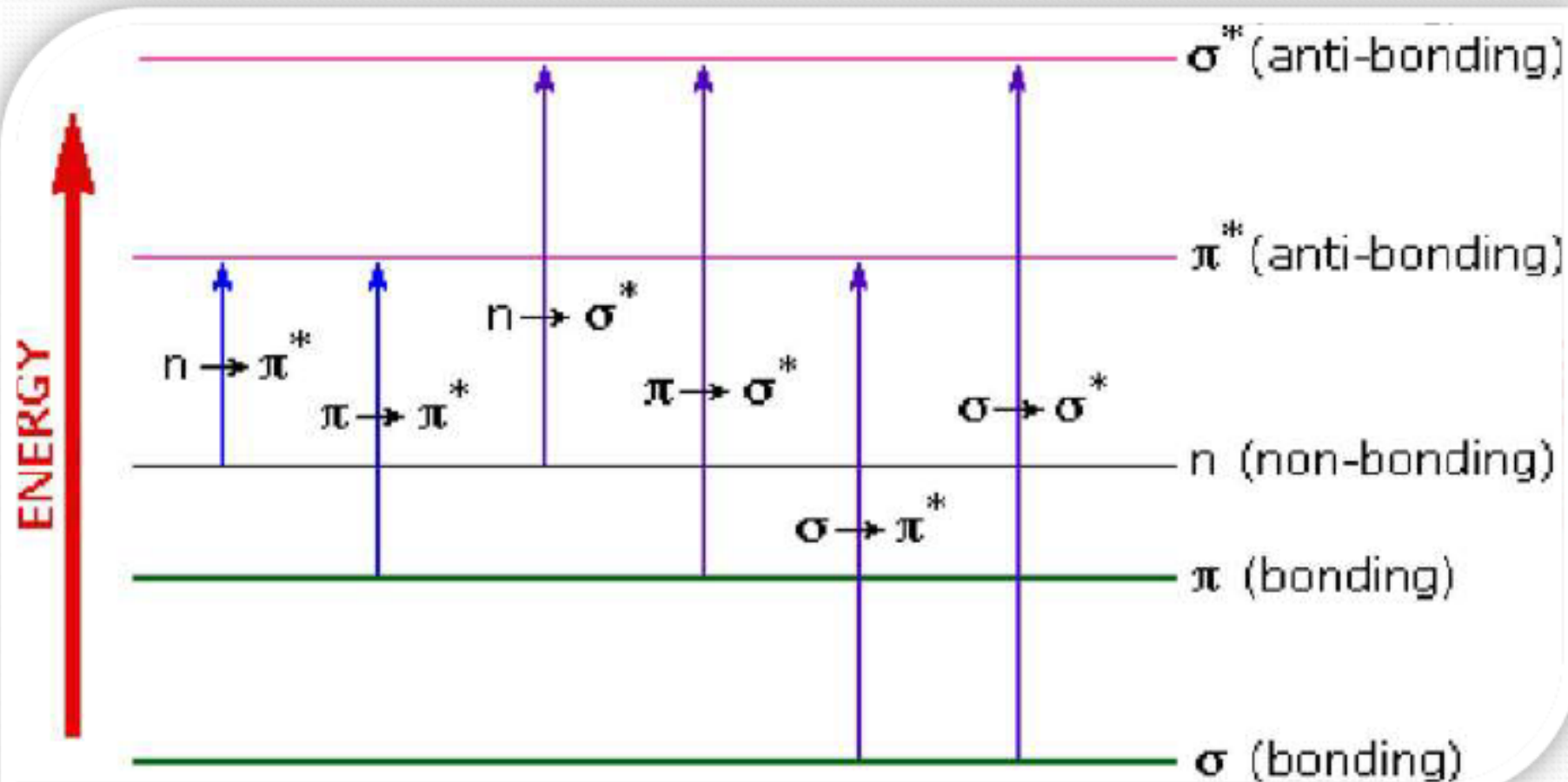
&

- $\pi \rightarrow \sigma^*$ transition

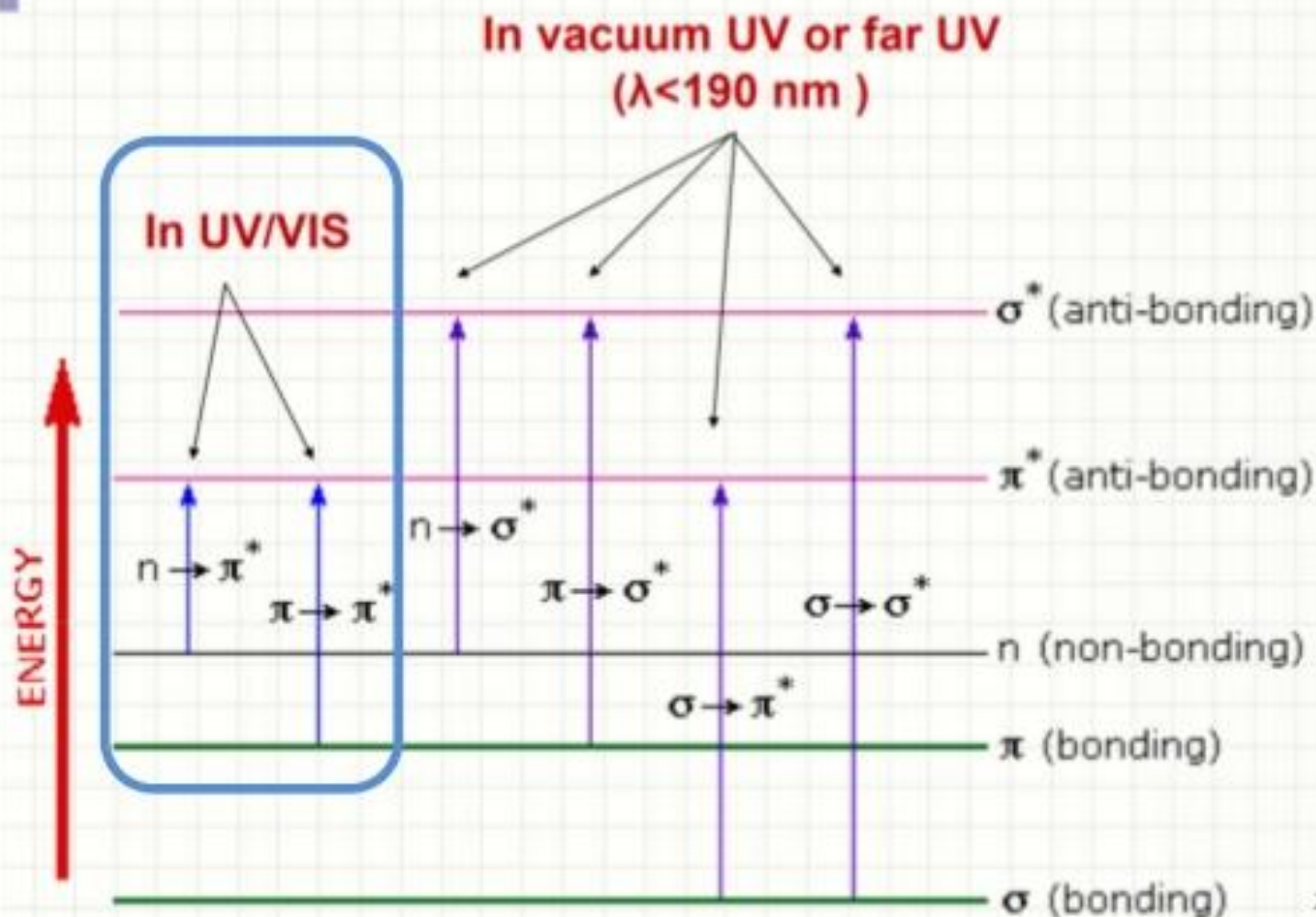
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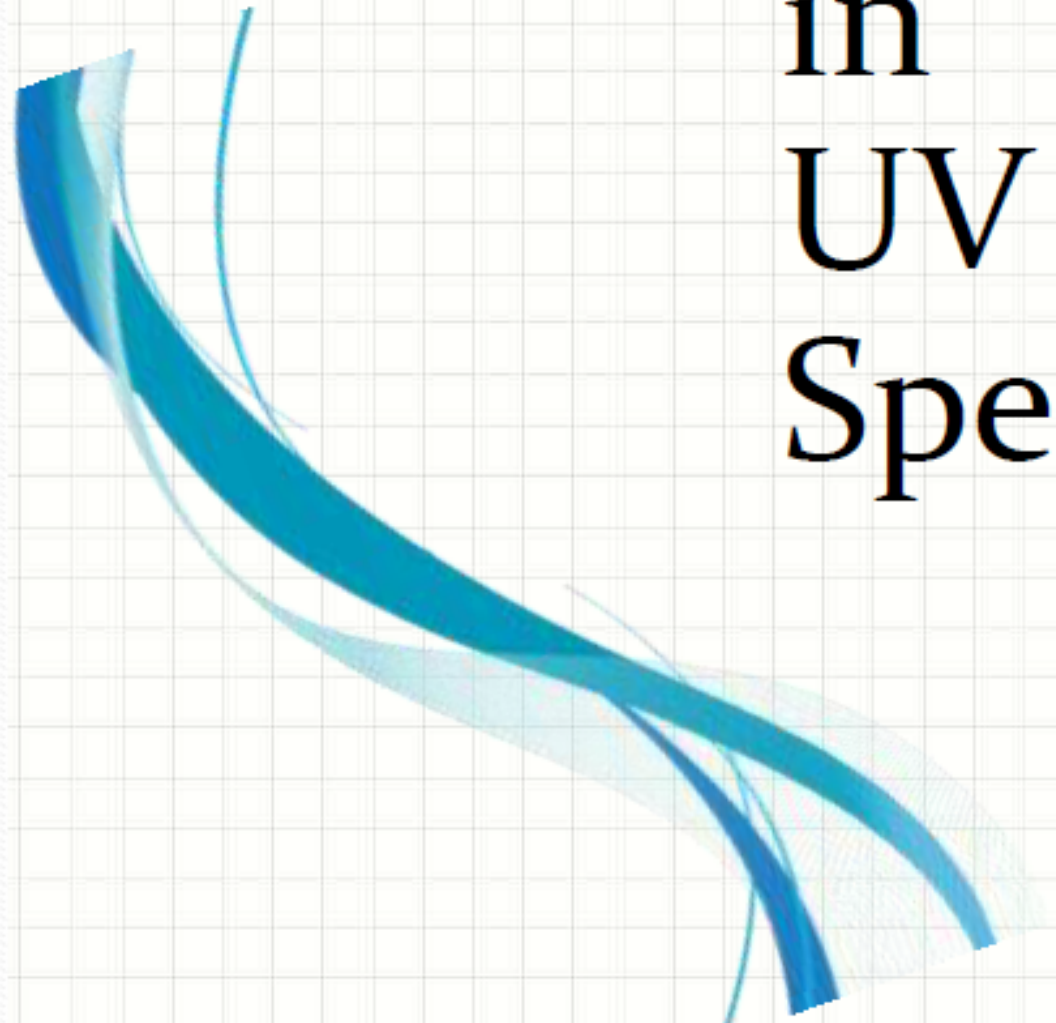
- These electronic transitions are forbidden transitions & are only theoretically possible.
- Thus, $n \rightarrow \pi^*$ & $\pi \rightarrow \pi^*$ electronic transitions show absorption in region above 200 nm which is accessible to UV-visible spectrophotometer.
- The UV spectrum is of only a few broad of absorption.

The possible electronic transitions can graphically shown as:



Possible electronic transitions





Terms used in UV / Visible Spectroscopy

Chromophore

The part of a molecule responsible for imparting color, are called as chromospheres.

OR

The functional groups containing multiple bonds capable of absorbing radiations above 200 nm due to $n \rightarrow \pi^*$ & $\pi \rightarrow \pi^*$ transitions.

e.g. NO_2 , $\text{N}=\text{O}$, $\text{C}=\text{O}$, $\text{C}=\text{N}$, $\text{C}\equiv\text{N}$, $\text{C}=\text{C}$, $\text{C}=\text{S}$, etc

Auxochrome

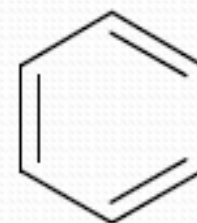
The functional groups attached to a chromophore which modifies the ability of the chromophore to absorb light, altering the wavelength or intensity of absorption.

OR

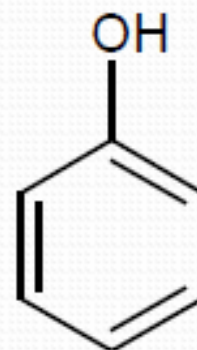
The functional group with non-bonding electrons that does not absorb radiation in near UV region but when attached to a chromophore alters the wavelength & intensity of absorption.

Auxochrome

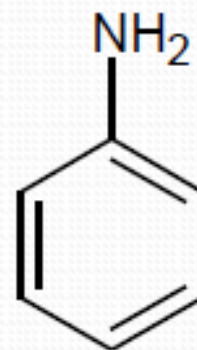
e.g. Benzene $\lambda_{\max} = 255 \text{ nm}$



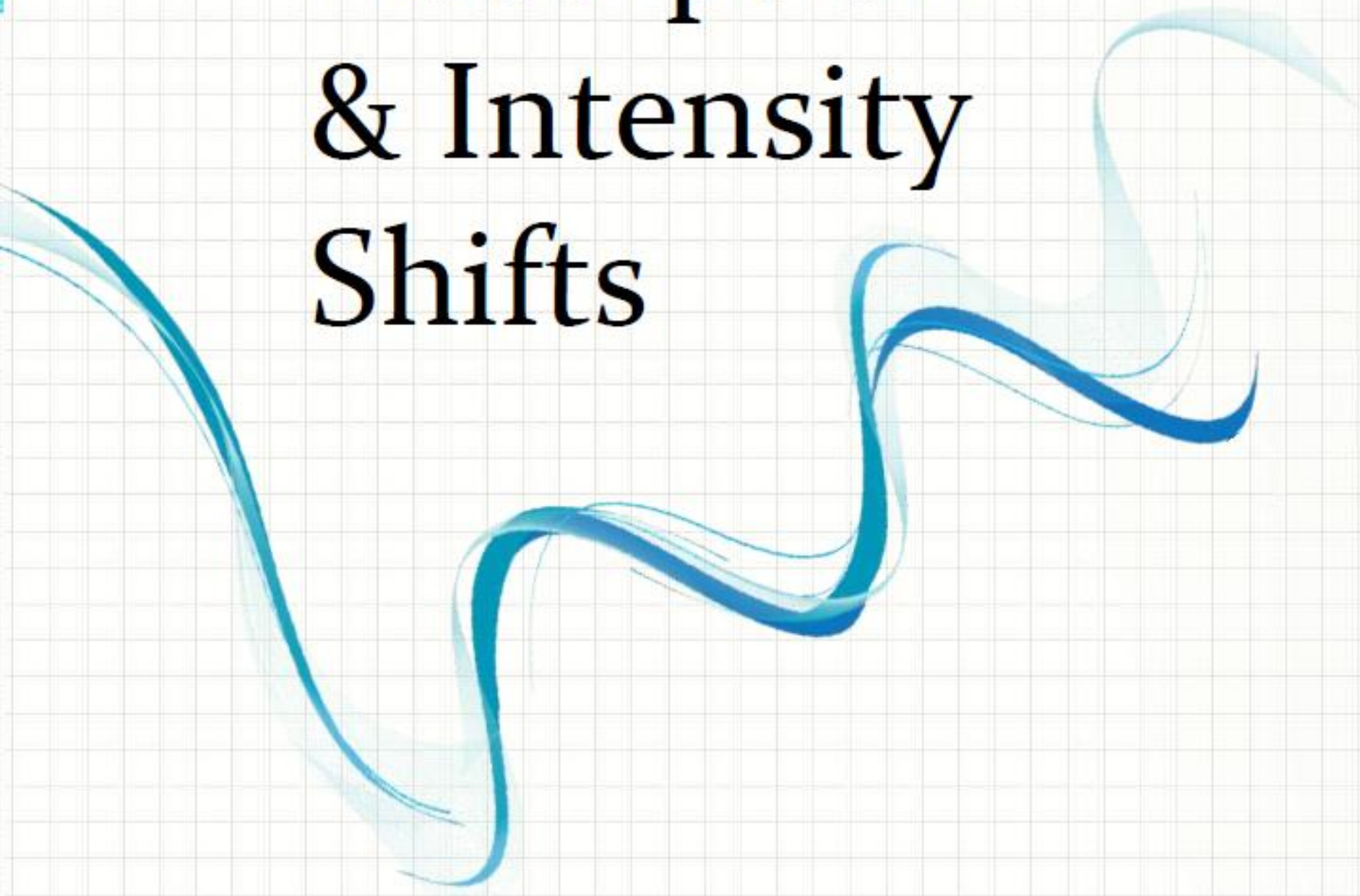
Phenol $\lambda_{\max} = 270 \text{ nm}$



Aniline $\lambda_{\max} = 280 \text{ nm}$



Absorption & Intensity Shifts



1

• Bathochromic Shift (Red Shift)

2

• Hypsochromic Shift (Blue Shift)

3

• Hyperchromic Effect

4

• Hypochromic Effect

1

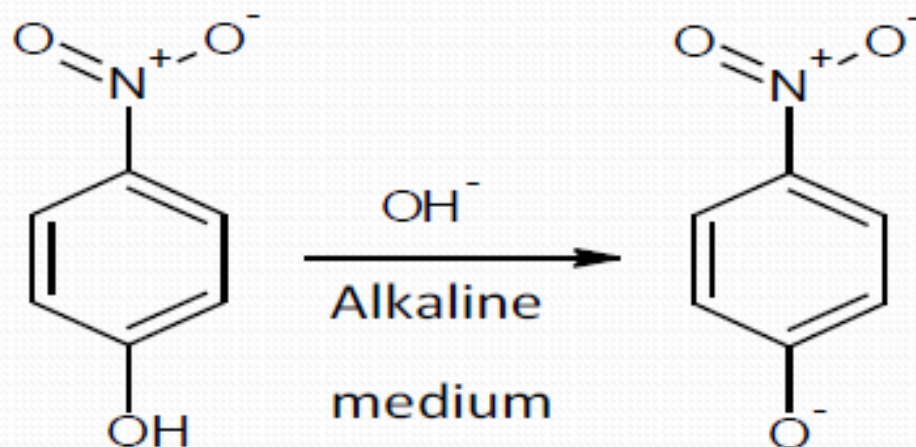
• Bathochromic Shift (Red Shift)

- When absorption maxima (λ_{\max}) of a compound shifts to longer wavelength, it is known as bathochromic shift or red shift.
- The effect is due to presence of an auxochrome or by the change of solvent.
- e.g. An auxochrome group like $-\text{OH}$, $-\text{OCH}_3$ causes absorption of compound at longer wavelength.

1

• Bathochromic Shift (Red Shift)

- In alkaline medium, p-nitrophenol shows red shift. Because negatively charged oxygen delocalizes more effectively than the unshared pair of electron.



p-nitrophenol

$\lambda_{\max} = 255 \text{ nm}$

$\lambda_{\max} = 265 \text{ nm}$

2

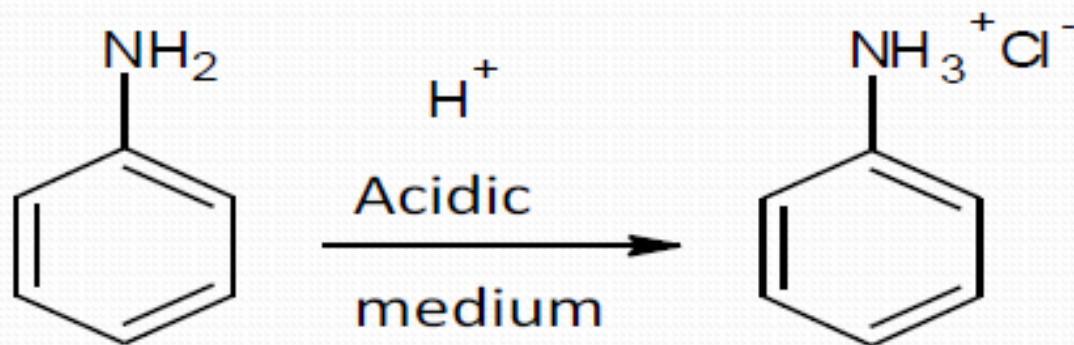
• Hypsochromic Shift (Blue Shift)

- When absorption maxima (λ_{max}) of a compound shifts to shorter wavelength, it is known as hypsochromic shift or blue shift.
- The effect is due to presence of an group causes removal of conjugation or by the change of solvent.

2

• Hypsochromic Shift (Blue Shift)

- Aniline shows blue shift in acidic medium, it loses conjugation.



Aniline

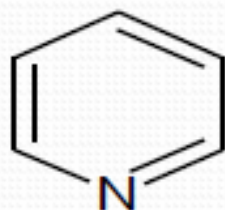
$\lambda_{\text{max}} = 280 \text{ nm}$

$\lambda_{\text{max}} = 265 \text{ nm}$

3

• Hyperchromic Effect

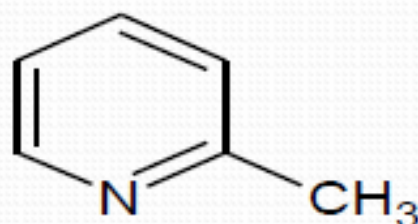
- When absorption intensity (ϵ) of a compound is increased, it is known as hyperchromic shift.
- If auxochrome introduces to the compound, the intensity of absorption increases.



Pyridine

$$\lambda_{\max} = 257 \text{ nm}$$

$$\epsilon = 2750$$



2methylpyridine

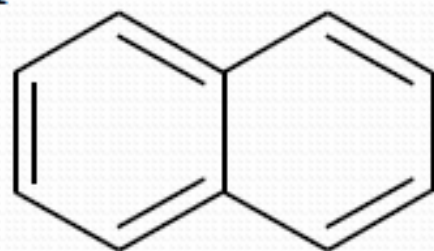
$$\lambda_{\max} = 260 \text{ nm}$$

$$\epsilon = 3560$$

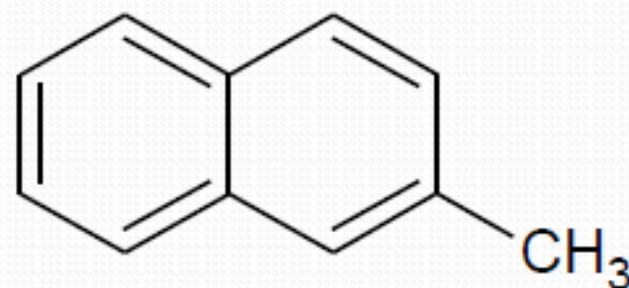
4

• Hypochromic Effect

- When absorption intensity (ϵ) of a compound is decreased, it is known as hypochromic shift.

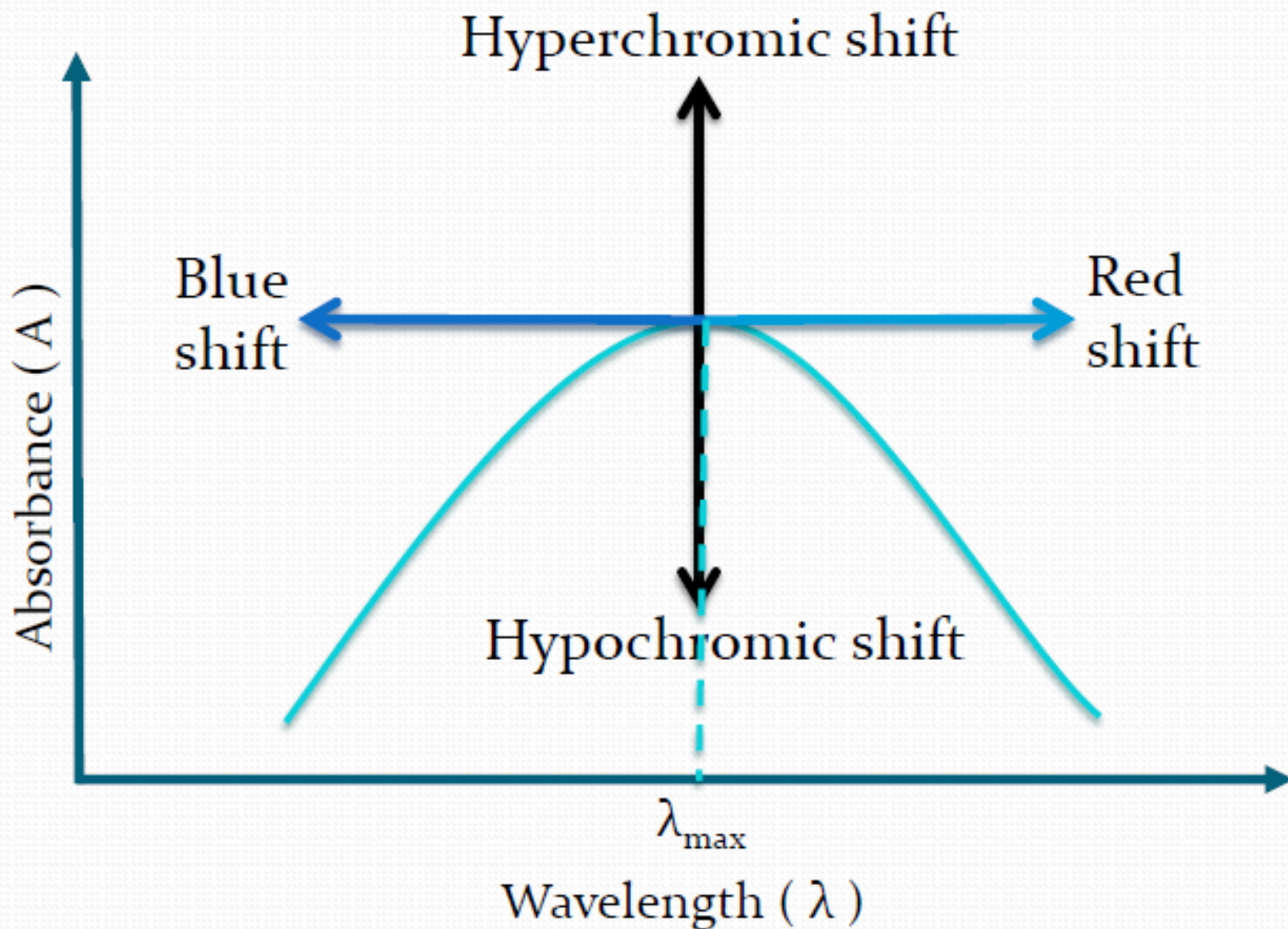



Naphthalene
naphthalene
 $\epsilon = 19000$



2-methyl
naphthalene
 $\epsilon = 10250$

Shifts and Effects





**PRINCIPLES OF UV
- VISIBLE
SPECTROSCOPY**

Principle

- The UV radiation region extends from 10 nm to 400 nm and the visible radiation region extends from 400 nm to 800 nm.

Near UV Region: 200 nm to 400 nm

Far UV Region: below 200 nm

- Far UV spectroscopy is studied under vacuum condition.
- The common solvent used for preparing sample to be analyzed is either ethyl alcohol or hexane.

Instrumentation

Components of UV-Visible spectrophotometer

- **Source**
- **Filters & Monochromator**
- **Sample compartment**
- **Detector**
- **Recorder**

Five Basic Optical Instrument Components

- 1) Source – A stable source of radiant energy at the desired wavelength (or λ range).
- 2) Wavelength Selector – A device that isolates a restricted region of the EM spectrum used for measurement (monochromators, prisms & filters).
- 3) Sample Container – A transparent container used to hold the sample (cells, cuvettes, etc).
- 4) Detector/Photoelectric Transducer – Converts the radiant energy into a useable signal (usually electrical).
- 5) Signal Processor & Readout – Amplifies or attenuates the transduced signal and sends it to a readout device as a meter, digital readout, chart recorder, computer, etc.

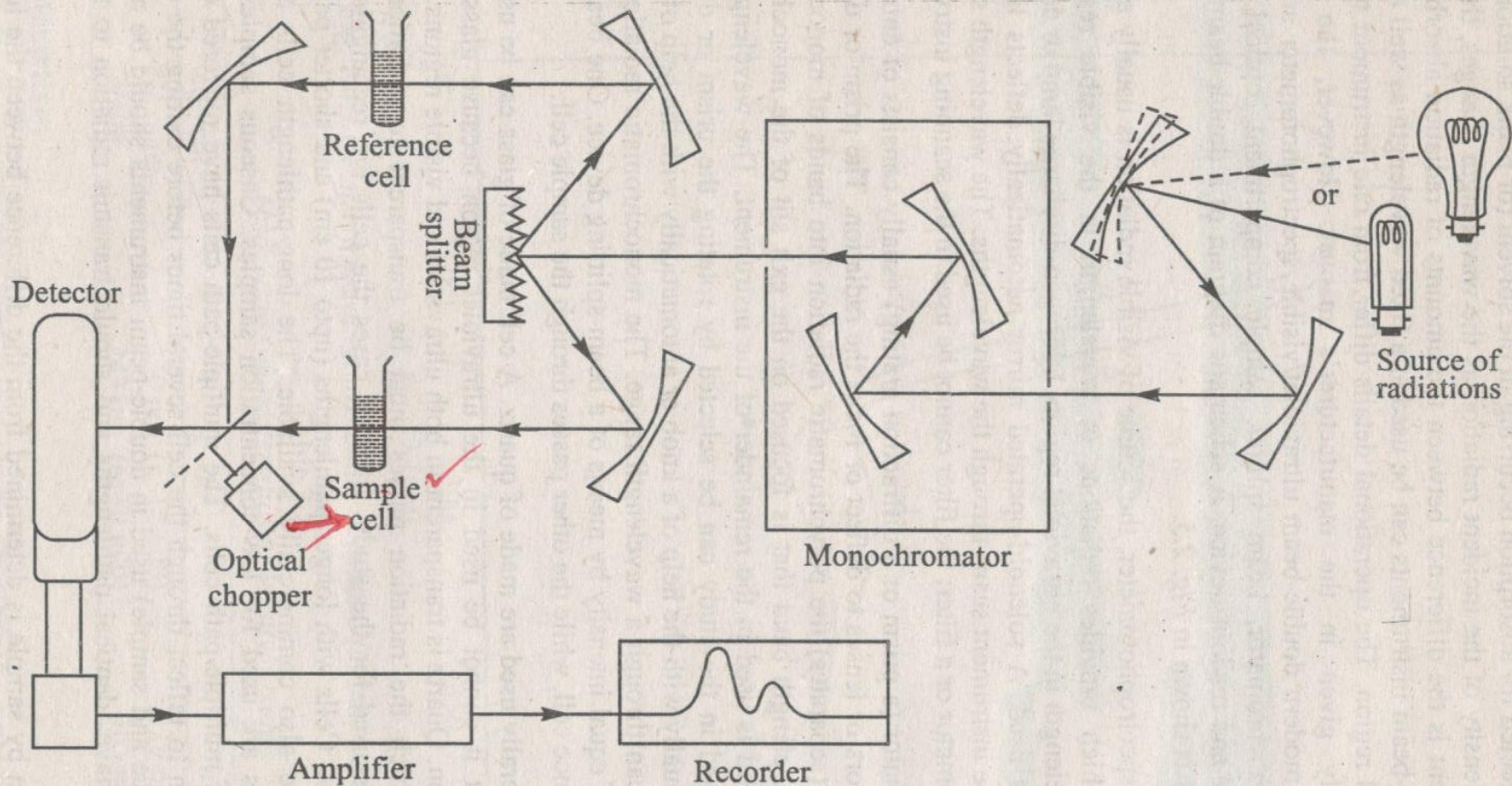


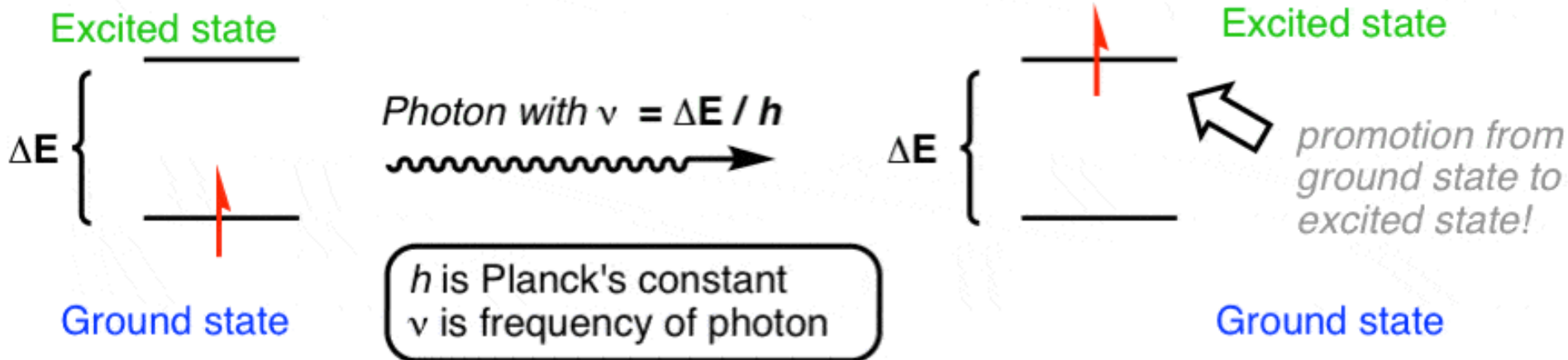
Fig. 2.5 Schematic diagram of a double-beam ultraviolet/visible spectrophotometer.

Conclusion:

- Qualitative & Quantitative Analysis:
 - It is used for characterizing aromatic compounds and conjugated olefins.
 - It can be used to find out molar concentration of the solute under study.
- Detection of impurities:
 - It is one of the important method to detect impurities in organic solvents.
- Detection of isomers are possible.
- Determination of molecular weight using Beer's law.

Summary: UV -Visible Spectroscopy

- Absorption of a photon with frequency ν can cause a transition between a ground state and excited state separated by energy ΔE



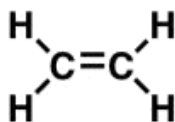
Since $E = h\nu$, absorption will occur when photon has frequency $\nu = \Delta E / h$

or since $c = \nu \lambda$, wavelength $\lambda = \frac{hc}{\Delta E}$
(c is speed of light)

- In molecules with pi (π) bonds, the energy gap ΔE between electrons in the ground state (π) and excited state (π^*) corresponds to photons in the ultraviolet (UV) and visible part of the electromagnetic spectrum (180 – 700 nm wavelength, or $E = 40 - 160$ kcal/mol)

- **Key Point:** As the number of **conjugated** (consecutive) π bonds increases, the energy gap (ΔE) **decreases**, meaning that light of less energy (**longer wavelength**) is absorbed.
- A **UV-Visible Spectrometer** lets us measure absorbance versus wavelength.
- The wavelength of maximum absorbance λ_{\max} corresponds to ΔE
- Therefore λ_{\max} **will be in the UV part of the spectrum for molecules with fewer conjugated π bonds and more toward the visible for molecules with many conjugated π bonds.**

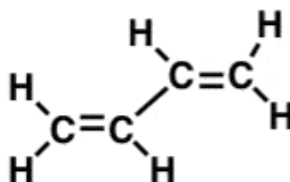
Ethene
("ethylene")



1 pi bond

λ_{\max} 180 nm
(UV)

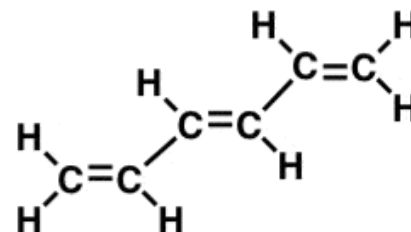
Butadiene
("1,3 butadiene")



2 conjugated pi bonds

λ_{\max} 217 nm
(UV)

Hexatriene
("1,3,5 hexatriene")



3 conjugated pi bonds

λ_{\max} 258 nm
(UV)

Fewer conjugated π bonds \rightarrow lower λ_{\max} (i.e. towards the UV)

More conjugated π bonds \rightarrow higher λ_{\max} (i.e. towards the visible)

Therefore using UV-Vis spectroscopy to determine the λ_{\max} of an unknown molecule can tell us about the π bonding present (if any)

Infrared spectroscopy

Infrared spectroscopy (IR spectroscopy or vibrational spectroscopy)

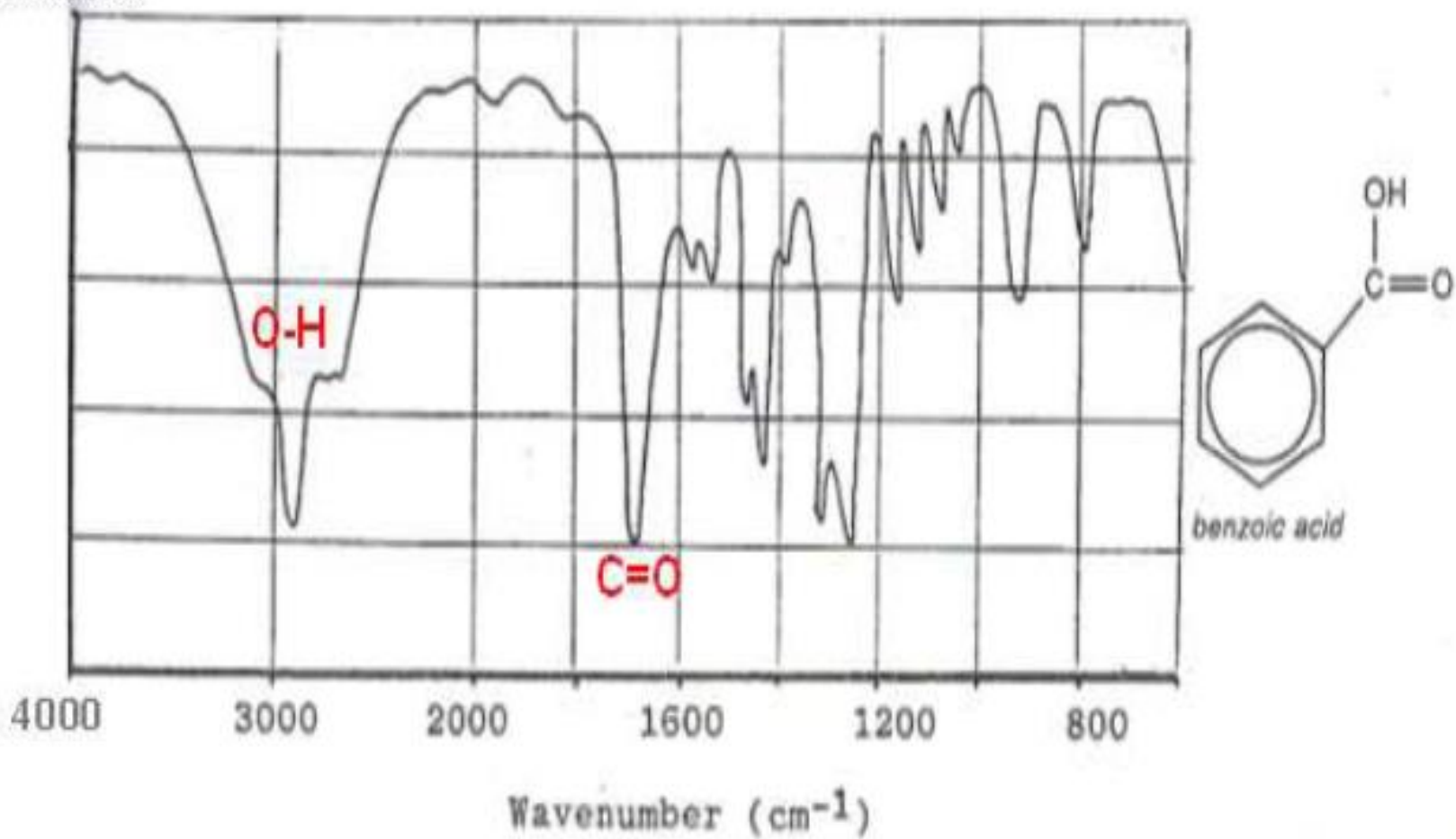
is the measurement of the interaction of **infrared** radiation with matter by absorption, emission, or reflection. It is used to study and identify chemical substances or functional groups in solid, liquid, or gaseous forms.

Principle of IR Spectroscopy

- When IR light is passed through a sample of organic compound some of the frequencies are absorbed while others are transmitted.
- Plot of Absorbance or Transmittance Vs Wave no. gives an IR spectrum
- But conventionally **Wave No. Vs % Transmittance** is plotted (because the numbers are more manageable)

The infrared spectrum of benzoic acid is shown below:

Transmittance

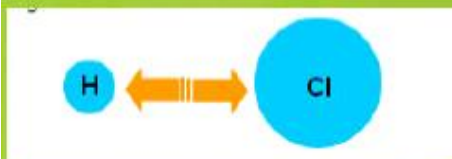


Requirements of IR radiation Absorption

1. Correct wavelength of radiation

If a molecule absorbs radiation only when natural frequency of vibration of some part of molecule is same as frequency of incident radiation.

2. Electric Dipole:



- If a frequency of vibration of HCl molecule exactly matches with that coming from the source **NET TRANSFER of energy takes place** → change in amplitude of molecular vibration → absorption of radiation
- In case of O₂, N₂, Cl₂ **NO NET change in dipole moment** occurs thus they cannot absorb IR radiations & do not show IR spectra

Two types changes occur in molecule :

- **Molecular rotations:**

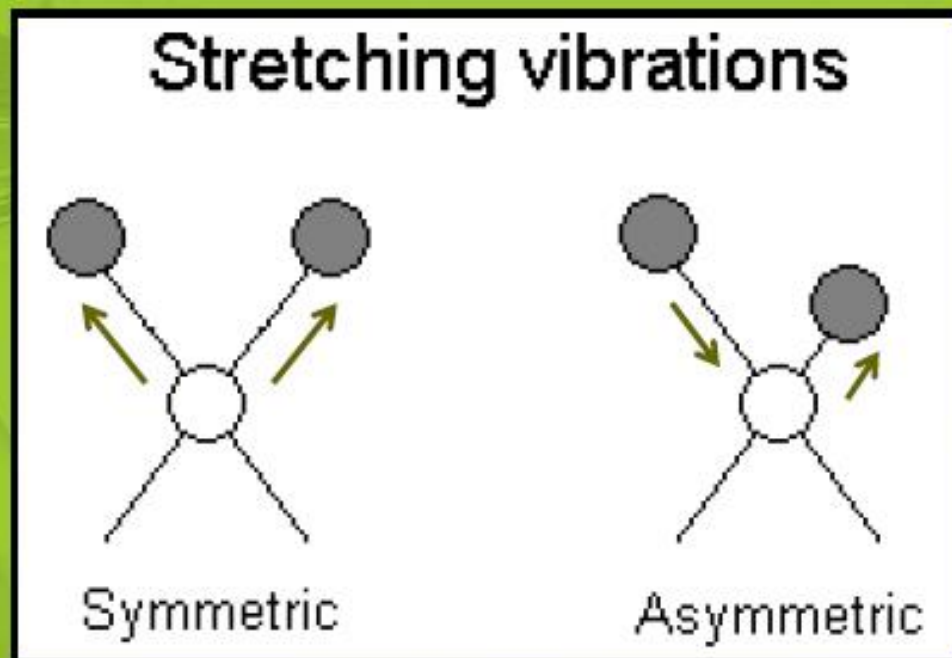
Rotational transitions are of little use to the spectroscopist.

- **Molecular vibrations**

The positions of atoms in a molecules are not fixed; they are subject to a number of different vibrations

TYPES OF MOELCULAR VIBRATIONS

Stretching: Change in inter-atomic distance along bond axis

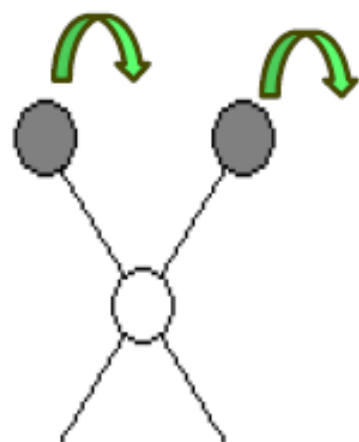


TYPES OF MOLECULAR VIBRATIONS

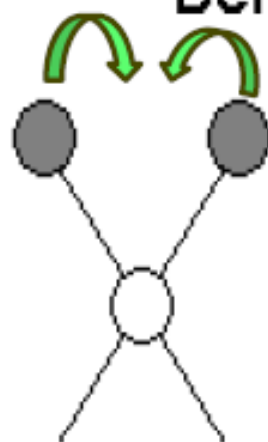
Bending: Change in angle between two bonds. There are four types of bend:

- Rocking
- Scissoring
- Wagging
- Twisting

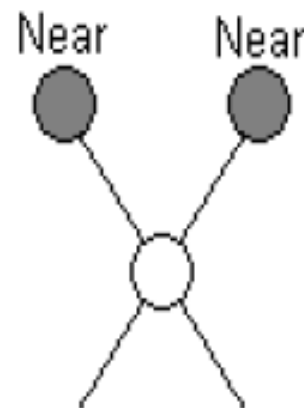
Bending vibrations



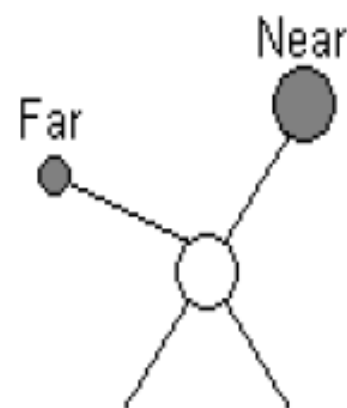
In-plane rocking



In-plane scissoring

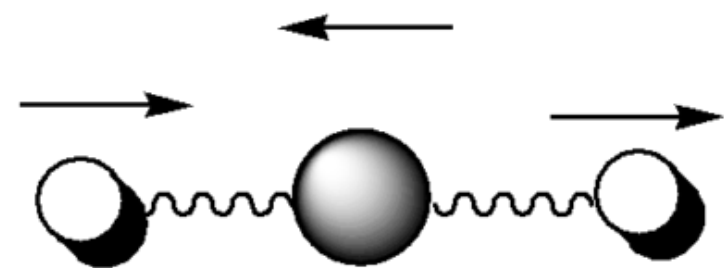


Out-of-plane wagging

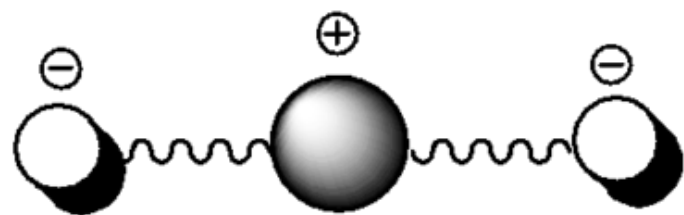


Out-of-plane twisting

CO₂ Molecule



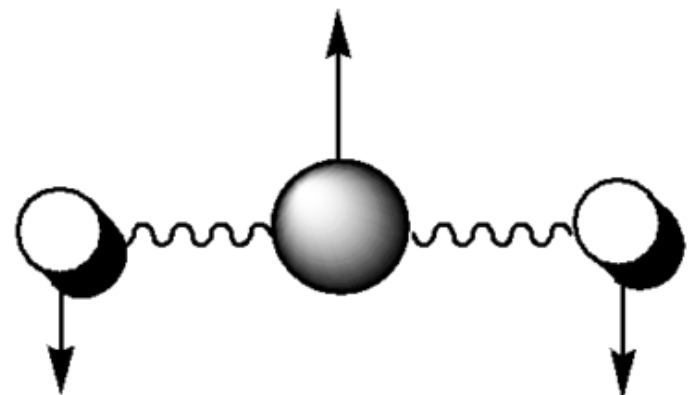
Asymmetrical stretching
~ 2350 cm⁻¹



Scissoring Bending
~665 cm⁻¹



Symmetrical stretching
~ 1340 cm⁻¹

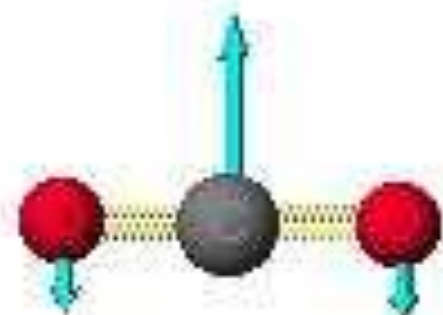


Scissoring Bending
~665 cm⁻¹

Carbon Dioxide - Infrared Absorption



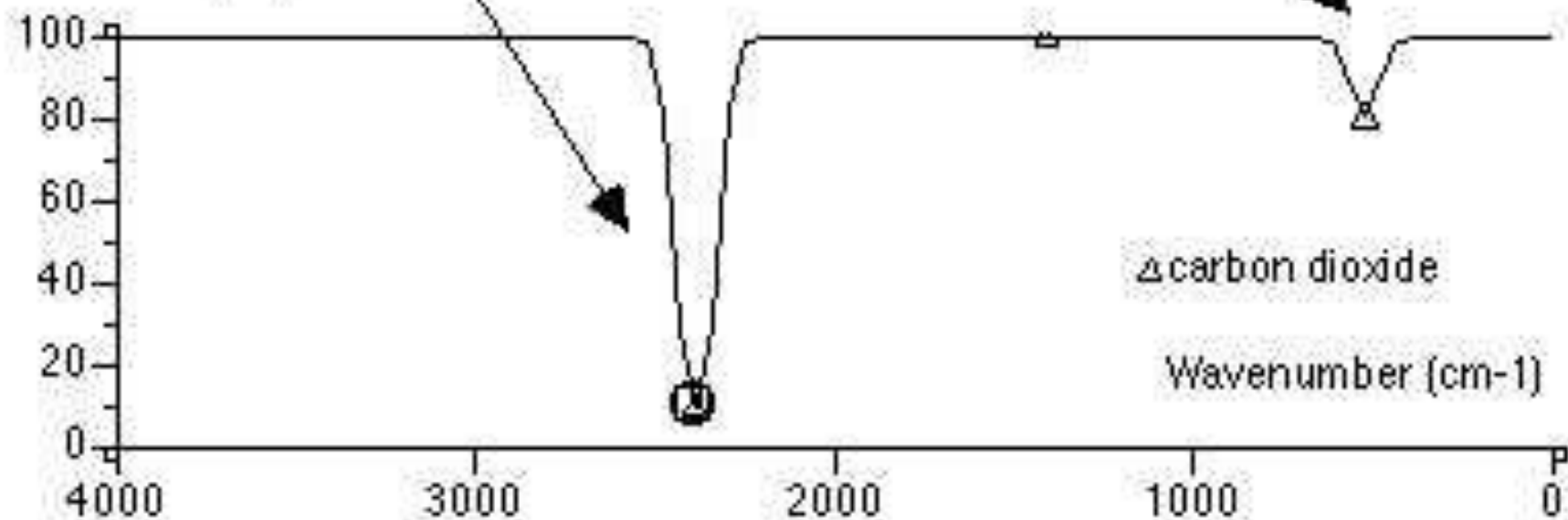
bond stretching



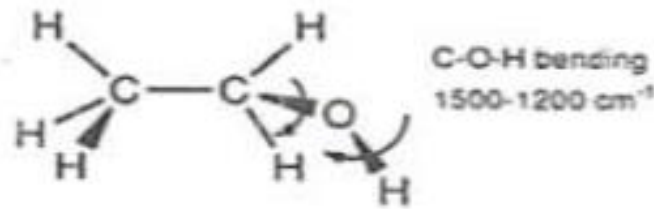
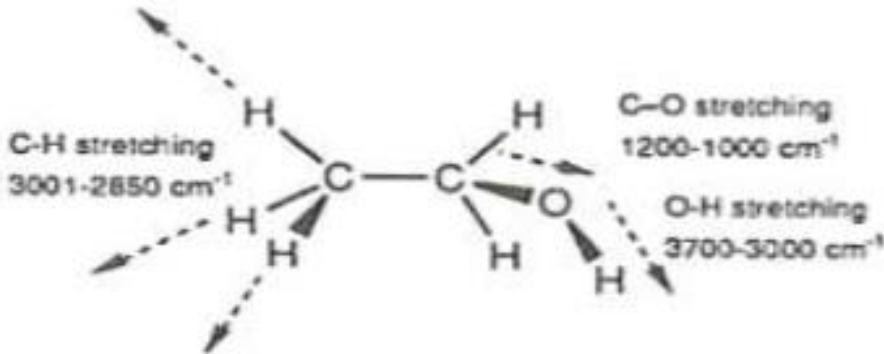
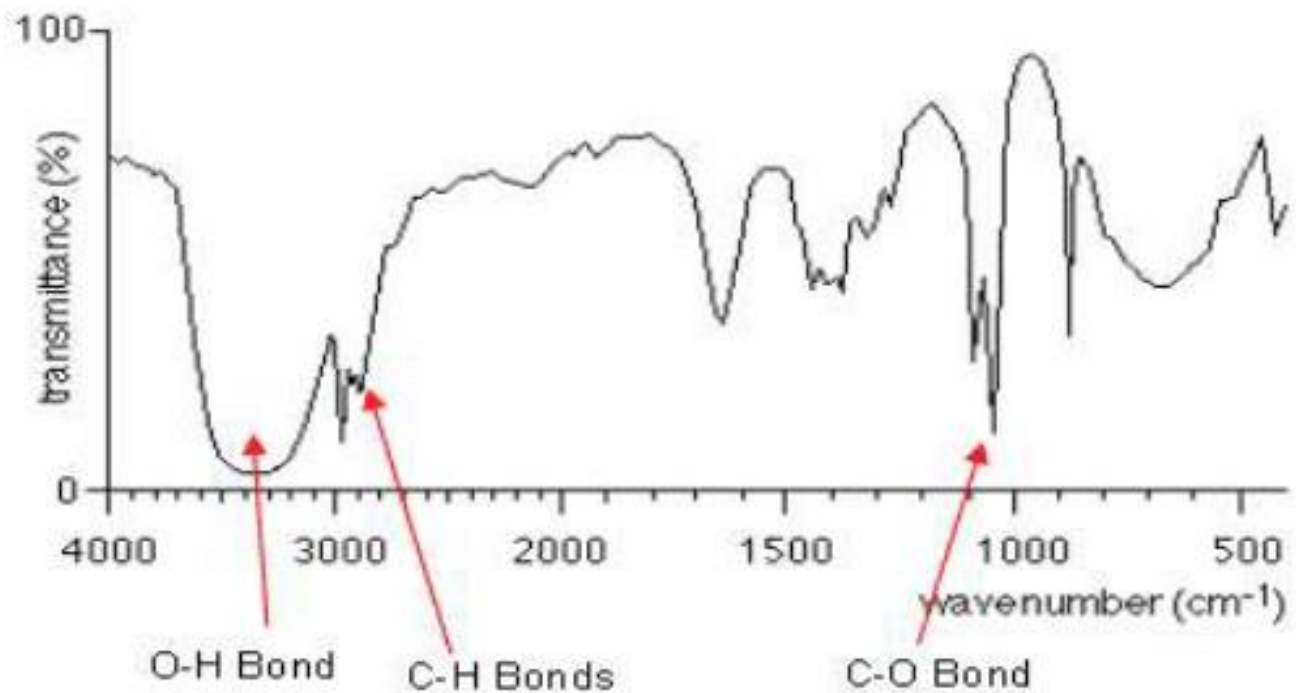
bond bending

Transmittance (%)

Vibrational Spectra



Below is the infrared spectrum of ethanol with some identified fingerprints:



Vibrational frequency

- A molecular vibration occurs when atoms in a molecule are in periodic motion while the molecule as a whole has constant translational and rotational motion. **The frequency of the periodic motion is known as a vibrational frequency**

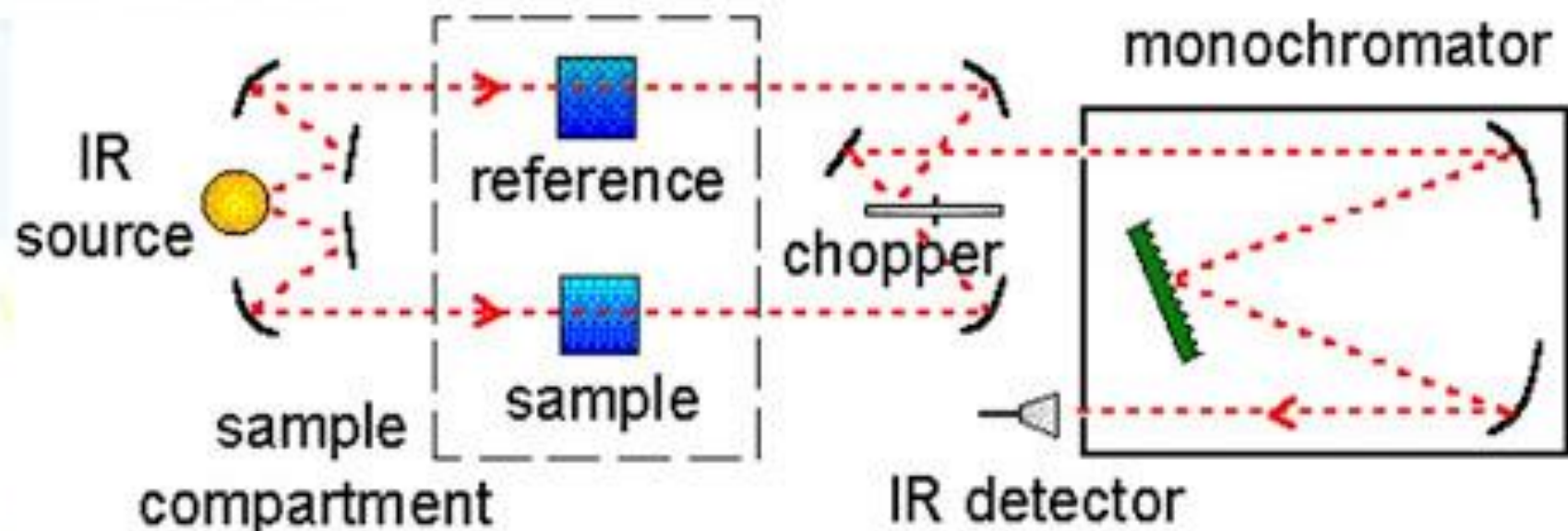
Factors affecting Vibrational frequency

- Vibrational coupling
- H-Bonding
- Electronic effect
- Field effect
- Nature of solvent

IR Instruments

Infrared Instruments

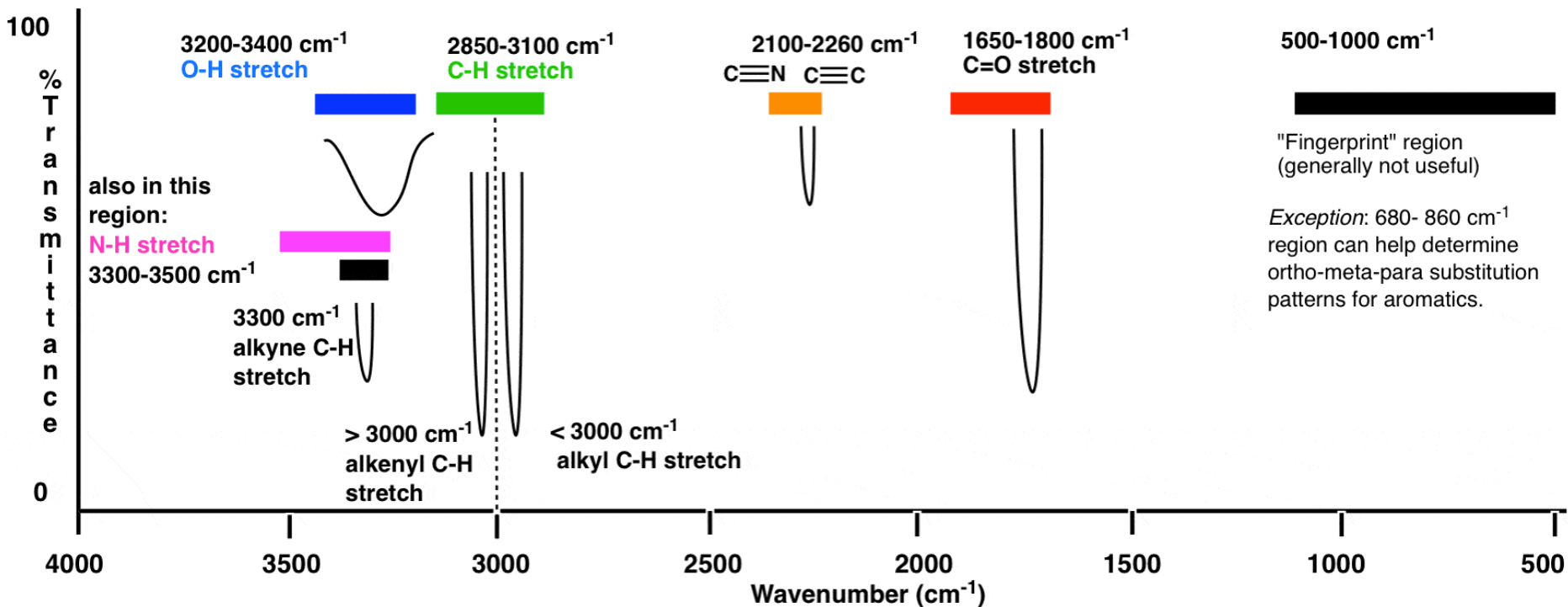
Schematic of a dispersive (double beam) IR spectrometer



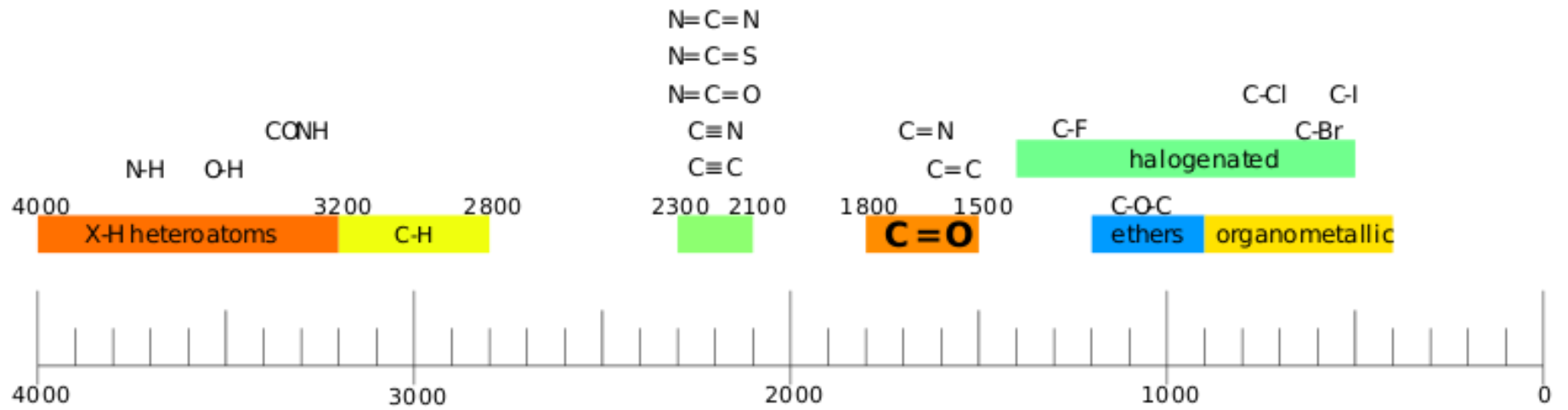
©1995 CHP

- Sample compartments **before the monochromator** (opposite for UV-vis) to diminish stray/scattered light problems. Possible because IR radiation does not tend to photodecompose compounds, unlike the UV/Vis

Typical Infrared Absorption Values For Various Types of Bonds



"Fingerprint" region (generally not useful)
Exception: 680- 860 cm⁻¹ region can help determine ortho-meta-para substitution patterns for aromatics.



Handling of samples in IR

- Sampling techniques in IR depend on whether the sample is a

- ✓ Gas - Gas cell

- ✓ Liquid - Thin film

- ✓ Solid - KBr disks

- Mulls

- Deposited films

Conventional
IR
Spectroscopy

Fourier
transform IR
Spectroscopy

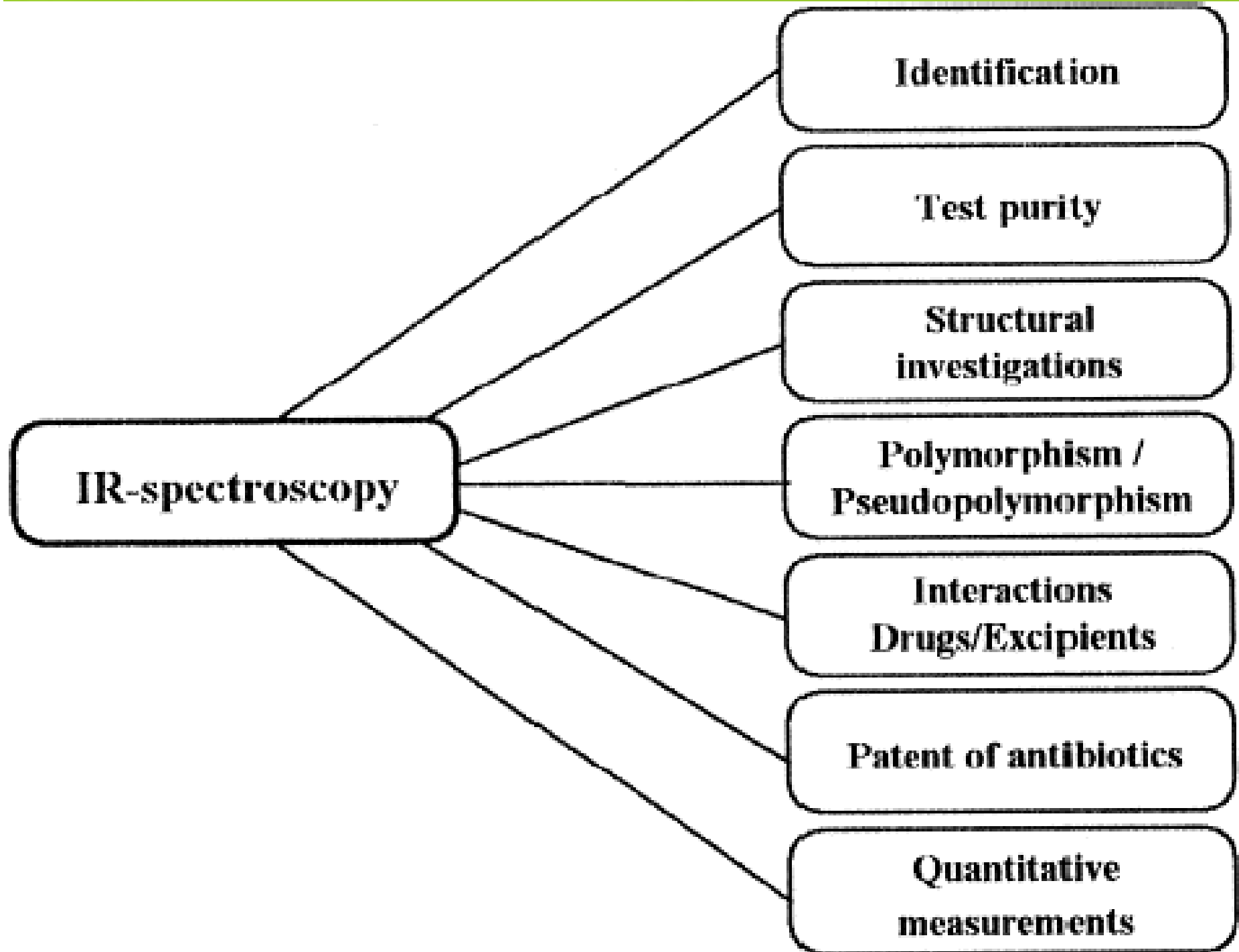
Dispersive or non
dispersive
instruments like
monochromator
and prisms

Frequency domain
spectra

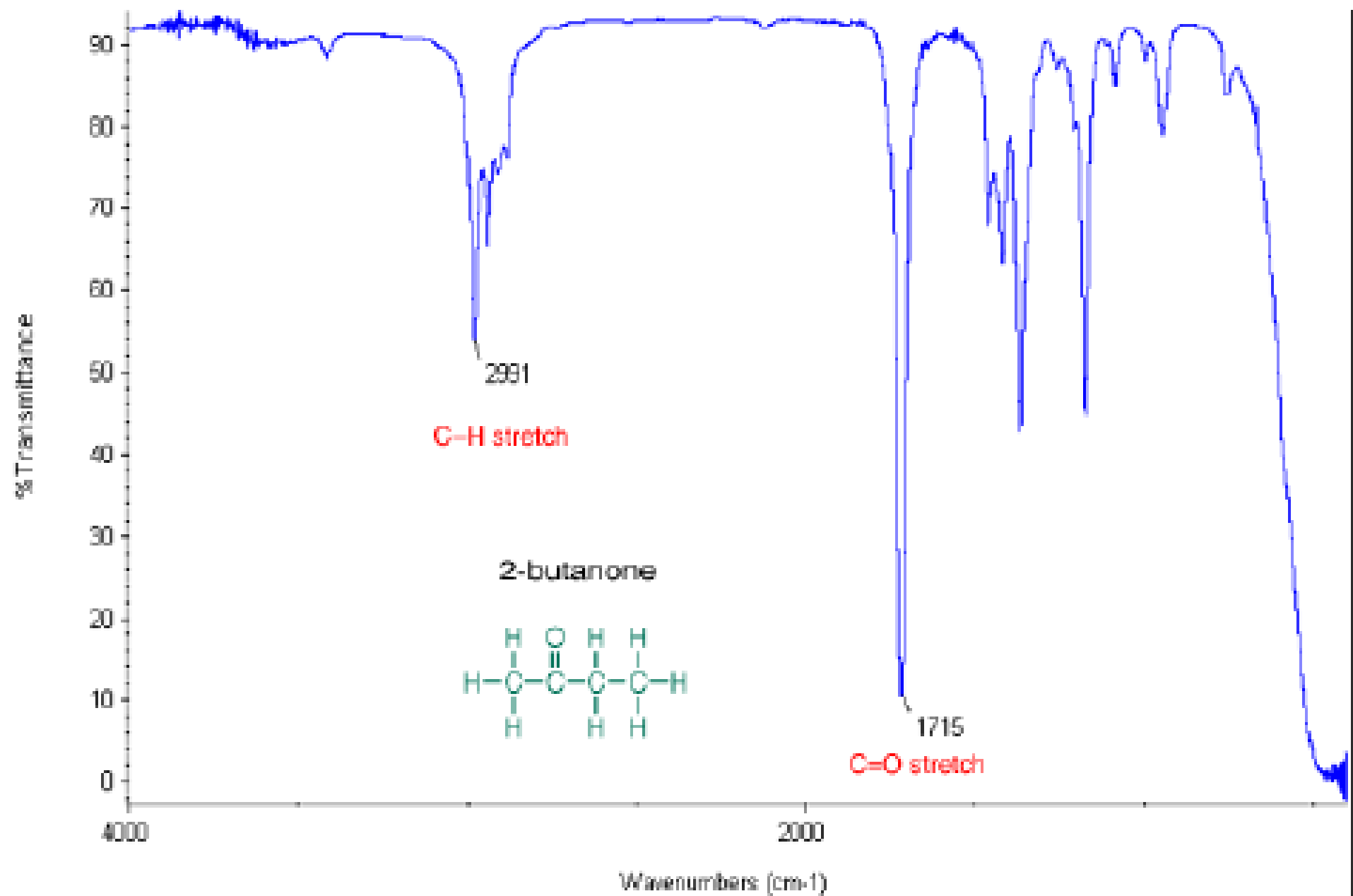
Interferometers
are used

Time domain
spectra

High signal to
noise ratio.



Ketones



Amines

