



**Republic of Iraq  
Ministry of Higher Education & Scientific research  
Al-Mustaqlal University  
Science College  
Biochemistry Department**

**Analytical Chemistry Instrumental Analysis**

**For  
Second Year Student/course 1  
Lecture 2**

**By  
Dr. Karrar M. Obaid**

**2025-2026**

## **Spectroscopic Methods**

- The spectroscopic methods of analysis are the most frequently employed technique in analysis .
- Many substances interact with electromagnetic radiation “Light” .
- Most of the spectroscopic methods are based on the fact that molecules are capable of absorbing radiant energy .
- The spectroscopic methods of analysis involve the measurement of the amount of light absorbed by the substance in solution .
- Spectrophotometry is used in clinical , industrial, educational and research areas for the analysis of drugs , food , beverages ,water and body fluids .

### **•The most spectroscopic methods of analysis are :**

- Ultraviolet – Visible (UV-)method
- Infrared ( IR-)method
- Fluorometric method
- Nuclear Magnetic Resonance (NMR) method
- Mass spectroscopic method
- Polarographic method.

**The importance of the spectrometric methods of analysis :**

- 1 - Qualitative and quantitative analysis of many compounds.
- 2 - Structure elucidation of organic compounds
- 3 - Studying the stability of drugs .
- 4 - Studying the kinetic of drugs .

### **Prefixes used with units**

<b>Prefix</b>	<b>Symbol</b>	<b>Meaning (Power of 10)</b>	<b>Example</b>
<b>deci</b>	d	$10^{-1}$	1 decimeter (dm) = 0.1 m
<b>centi</b>	c	$10^{-2}$	1 centimeter (cm) = 0.01 m
<b>milli</b>	m	$10^{-3}$	1 millimeter (mm) = 0.001 m
<b>micro</b>	$\mu$	$10^{-6}$	1 micrometer ( $\mu$ m) = $1 \times 10^{-6}$ m
<b>nano</b>	n	$10^{-9}$	1 nanometer (nm) = $1 \times 10^{-9}$ m
<b>pico</b>	p	$10^{-12}$	1 picometer (pm) = $1 \times 10^{-12}$ m

### **Nature of electromagnetic radiation**

- 1- Electromagnetic radiation is a form of radiant energy such as sun light , radio waves and x-rays .
- 2- The white light can be split to produce different colors or wavelengths .

3- The visible spectrum forms only a small part of the complete spectrum of electro-magnetic radiation which extends from the ultra short wave region rays at one end to that of the radio-waves at the other end .

4- The visible region of the spectrum extends from 380 nm to 750 nm .

Wavelength Range Absorbed	Colour Absorbed	Colour Seen By Eye
380 - 430	Violet	Yellow - Green
430 - 480	Blue	Yellow
480 - 490	Green - Blue	Orange
490 – 500	Blue - Green	Red
500 - 560	Green	Purple
560 - 580	Yellow - Green	Violet
580 - 590	Yellow	Blue
590 - 610	Orange	Green - Blue
610 - 750	Red	Blue - Green

- ✓ Ultraviolet ( UV ) means “beyond the violet” . UV radiation has shorter wavelengths than violet light and cannot be seen by the eye .
- ✓ Infrared ( IR ) means “ below the red . IR radiation has longer wavelengths than red and also cannot be seen by the eye .

- ✓ When all the wavelengths or colors of the visible light are transmitted together, the light appears as white light , if all wavelengths or colors of the visible light are absorbed , it appears black .
- ✓ Colored substances appear colored because they selectively absorb some of the wavelengths of visible light and transmitted other wavelengths or colors ( apparent color . For example , red substance absorb blue - green wavelengths from the visible region, so the transmitted light appears red , blue substance absorb the yellow wavelengths , so the transmitted light appears blue .

## **Properties of electromagnetic radiation**

Electromagnetic radiation consists of oscillating electric and magnetic fields that propagate through space along a linear path and with a constant velocity.

### **a - Wave properties :**

such as reflection , refraction , scattering ,polarisation .

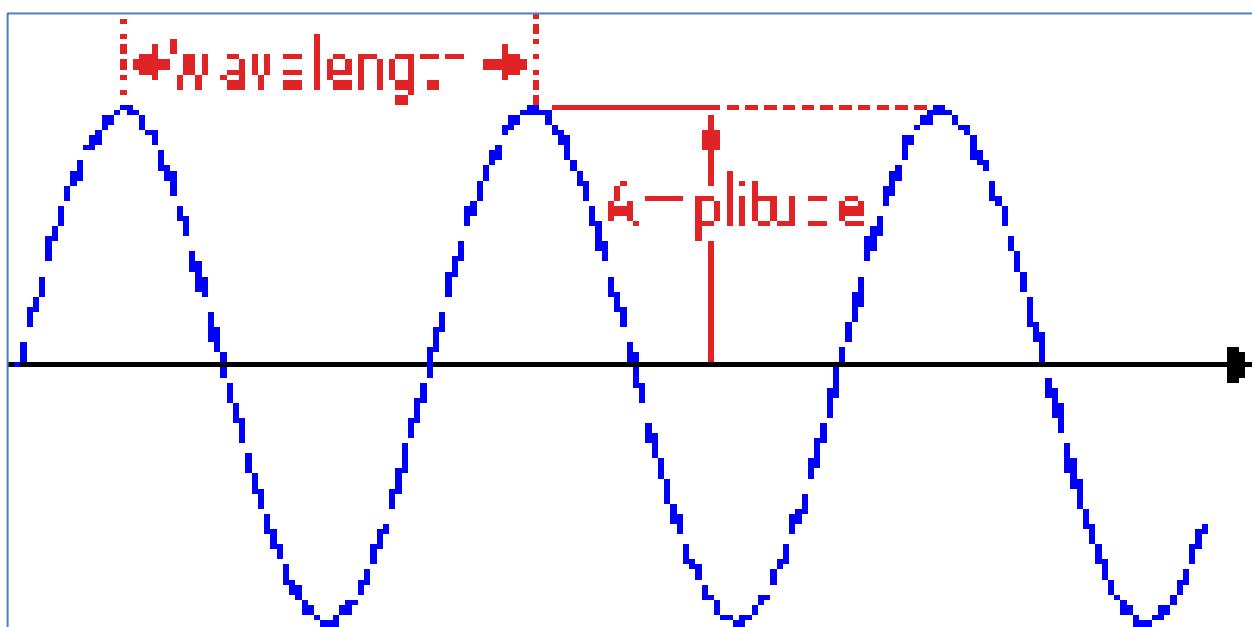
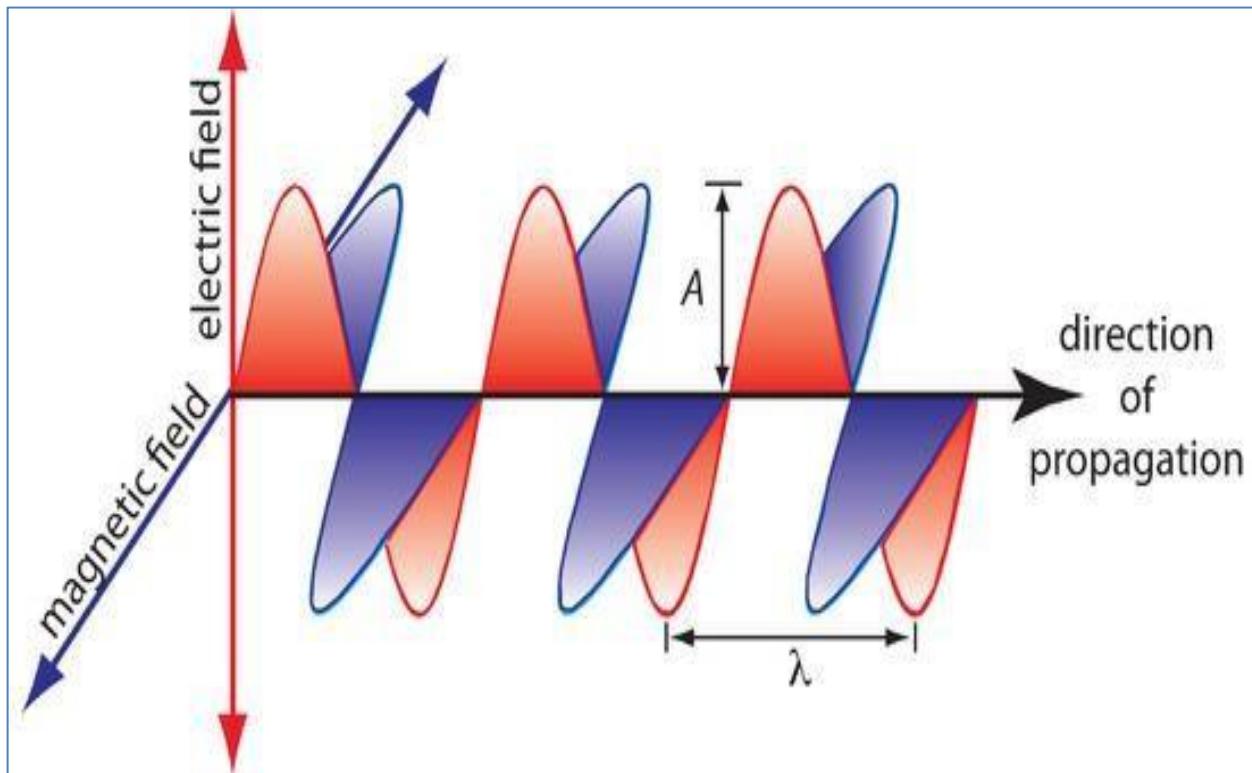
**Wavelength** : is the distance between any two successive points on the wave ( two successive maxima or minima of a wave ) nm . ( $\lambda = c/v$  )( $c=3 \times 10^8$  m/s)

**Wavenumber** : is the reciprocal of the wavelength (  $1/\lambda$  ) in  $\text{cm}^{-1}$  , which is the number of waves per 1 cm.

**Frequenvy**  $v$  : is the number of waves emitted per second in Hz (cycle / s ) .

**wavenumber**  $v'$  = 1 / wavelength (cm)

$$= \text{frequency} / \text{speed of light} = 1 / \lambda = v / c \text{ (cm/second)}$$



## Particle properties :

- ✓ The interaction of EMR can be accounted for by the particle properties of light . This postulate can be used to illustrate absorption or emission of radiant energy . EMR behaves as it is a train of photons ; discrete wave packets of distinct particles .
- ✓ The energy of photon depends upon the frequency of the radiation

$$E = h \nu$$

where  $h$  is plank,s constant (  $6.6 \times 10^{-34}$  j.s,)

- ✓ The relation between the frequency of light and its wavelength reveals that a photon of high frequency ( short  $\lambda$  ) has high energy content than one of lower frequency ( longer  $\lambda$  ).  
from

$$c = \lambda \cdot \nu$$

$$\nu = c / \lambda$$

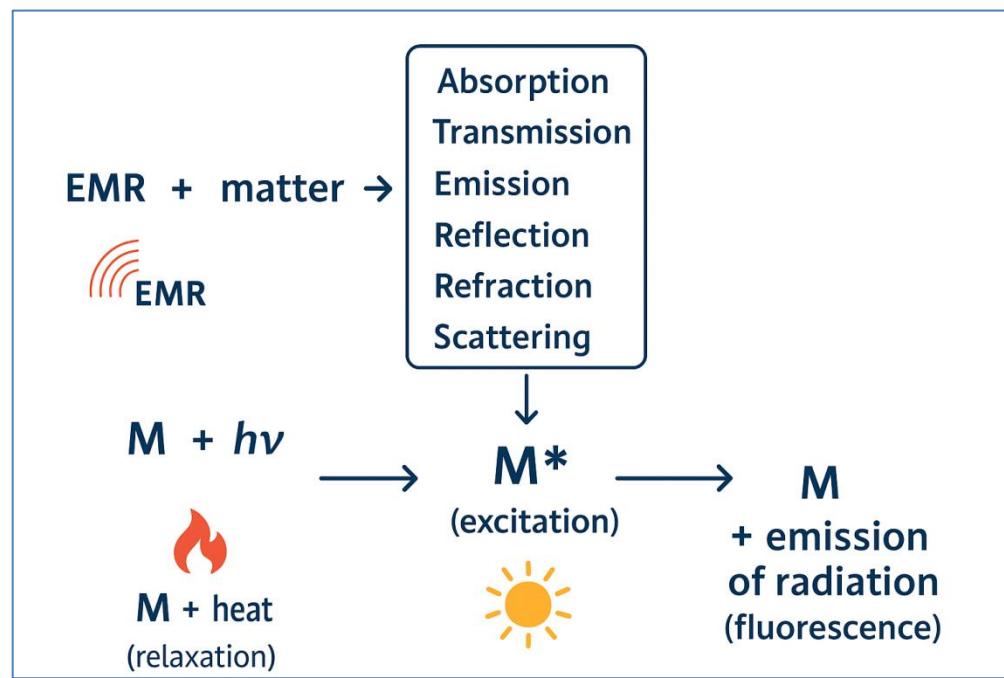
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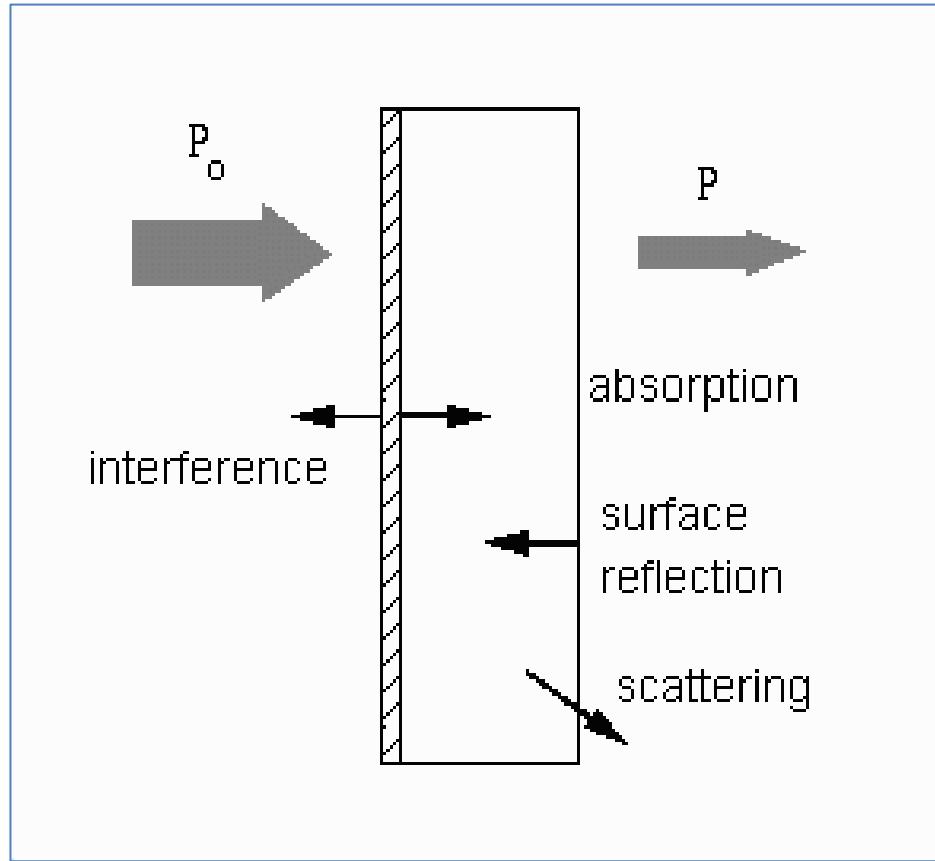
$$E = h \cdot c / \lambda$$

❖ The following table lists the names of different spectral regions, the range of frequencies and wavelengths in those regions, and the type of transition that can occur when a photon in these spectral ranges interacts with matter.

Type of radiation	Frequency(Hz)	Wavelength range	Type of Transition
gamma-rays	$10^{20}$ - $10^{24}$	<1 pm	nuclear
X-rays	$10^{17}$ - $10^{20}$	1 pm	inner electron
Ultraviolet	$10^{15}$ - $10^{17}$	400 nm-1 nm	outer electron
Visible	$4-7.5 \times 10^{14}$	750 nm-400 nm	outer electron
near-infrared vibrations	$1 \times 10^{14}$ - $4 \times 10^{14}$	2.5 μm-750 nm	outer electron molecular
Infrared	$10^{13}$ - $10^{14}$	25 μm-2.5 μm	molecular vibrations
Microwaves	$3 \times 10^{11}$ - $10^{13}$	1mm-25 μm	molecular rotations,
radio waves	< $3 \times 10^{11}$	>1 mm	nuclear spin flips*

## Absorption of Radiation





**The interaction of radiation with matter can cause :**

- ❖ Absorption:
- ❖ Transmission
- ❖ Emission:
- ❖ Scattering
- ❖ Reflection
- ❖ Refraction