



Republic of Iraq
Ministry of Higher Education & Scientific research
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Biochemistry Department

Analytical Chemistry Instrumental Analysis

For

Second Year Student/course 1

Lecture 2

By

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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

Prefix	Symbol	Meaning (Power of 10)	Example
deci	d	10^{-1}	1 decimeter (dm) = 0.1 m
centi	c	10^{-2}	1 centimeter (cm) = 0.01 m
milli	m	10^{-3}	1 millimeter (mm) = 0.001 m
micro	μ	10^{-6}	1 micrometer (μm) = 1×10^{-6} m
nano	n	10^{-9}	1 nanometer (nm) = 1×10^{-9} m
pico	p	10^{-12}	1 picometer (pm) = 1×10^{-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 .

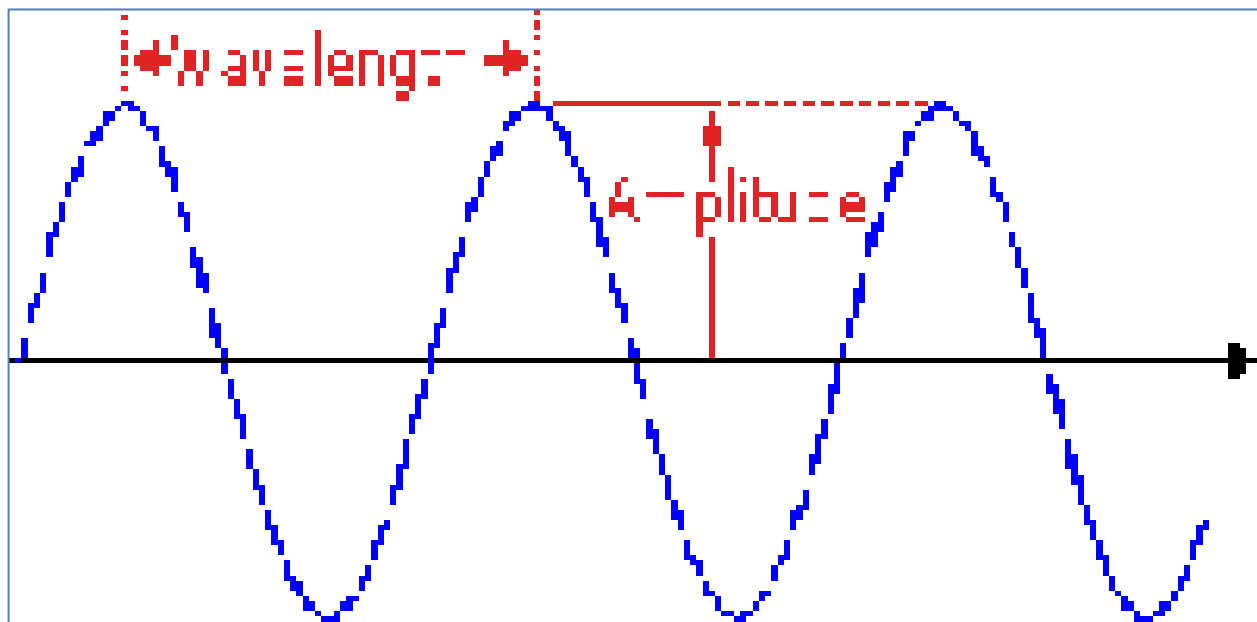
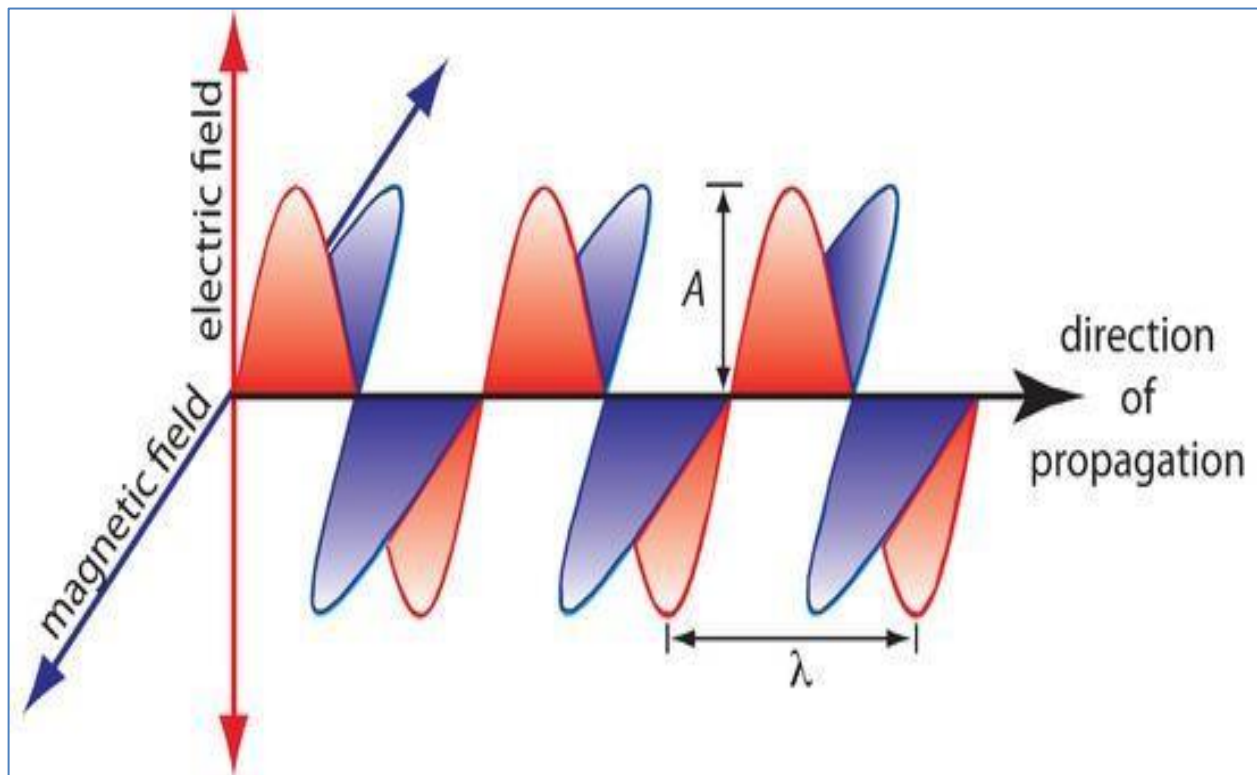
Wavelength : is the distance between any two successive points on the wave (two successive maxima or minima of a wave) nm . ($\lambda = c/v$)(c=3x10⁸ m/s)

Wavenumber : is the reciprocal of the wavelength ($1/\lambda$) in cm⁻¹ , which is the number of waves per 1 cm.

Frequency ν : is the number of waves emitted per second in Hz (cycle / s) .

wavenumber ν' = 1 / wavelength (cm)

= frequency / speed of light = $1 / \lambda = \nu / c$ (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 (6.6×10^{-34} j.s,)

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

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

$$\nu = c / \lambda$$

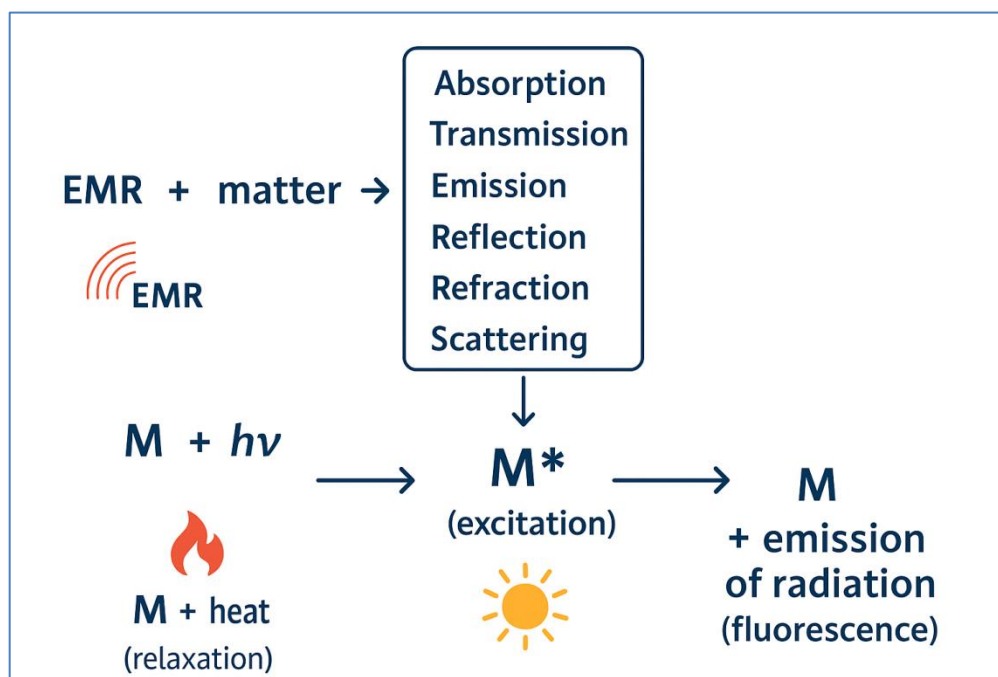
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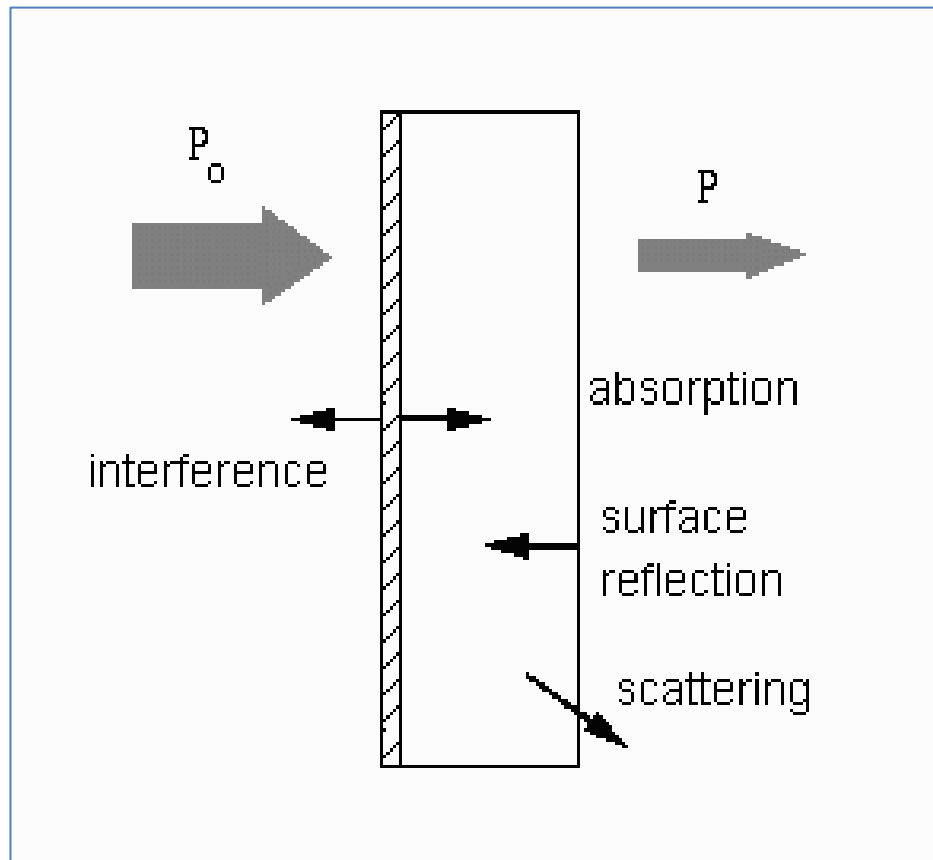
$$E = h \cdot c / \lambda$$

- ❖ 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 nm-	1 pm	inner electron
Ultraviolet	10^{15} - 10^{17}	400 nm-1 nm	outer electron
Visible	4 - 7.5×10^{14}	750 nm-400 nm	outer electron
near-infrared vibrations	1×10^{14} - 4×10^{14}	2.5 μ m-750 nm	outer electron molecular
Infrared	10^{13} - 10^{14}	25 μ m-2.5 μ m	molecular vibrations
Microwaves	3×10^{11} - 10^{13}	1mm-25 μ m	molecular rotations,
radio waves	< 3×10^{11}	>1 mm	nuclear spin flips*

Absorption of Radiation





The interaction of radiation with matter can cause :

- ❖ [Absorption](#):
- ❖ Transmission
- ❖ [Emission](#)..
- ❖ [Scattering](#)
- ❖ Reflection
- ❖ Refraction