Advance pharmaceutical analysis:

- The aim of this course to teach the students how to identify organic compounds from the:

synergistic information afforded by the combination of mass (MS), infrared (IR), nuclear magnetic resonance (NMR), and ultraviolet (UV) spectra. Essentially, the molecule is perturbed by these energy probes and the molecule's responses are recorded as spectra.

The pharmaceutical analyses remain unchanged, but remarkable evolution of instrumentation has been done.

In comparison, ultraviolet spectrometry has become relatively less useful for our purpose.

NMR, without question, has become the most sophisticated tool available to the organic chemist, in comparison; ultraviolet spectrometry has become relatively less useful for our purpose.

Mass spectrometry	Molecular size and formula
Infrared spectroscopy	Functional groups
NMR spectroscopy	Map of carbon–hydrogen framework

Absorption of UV-Visible light is chiefly caused by electronic excitation; the spectrum provides limited information about the structure of the molecule.

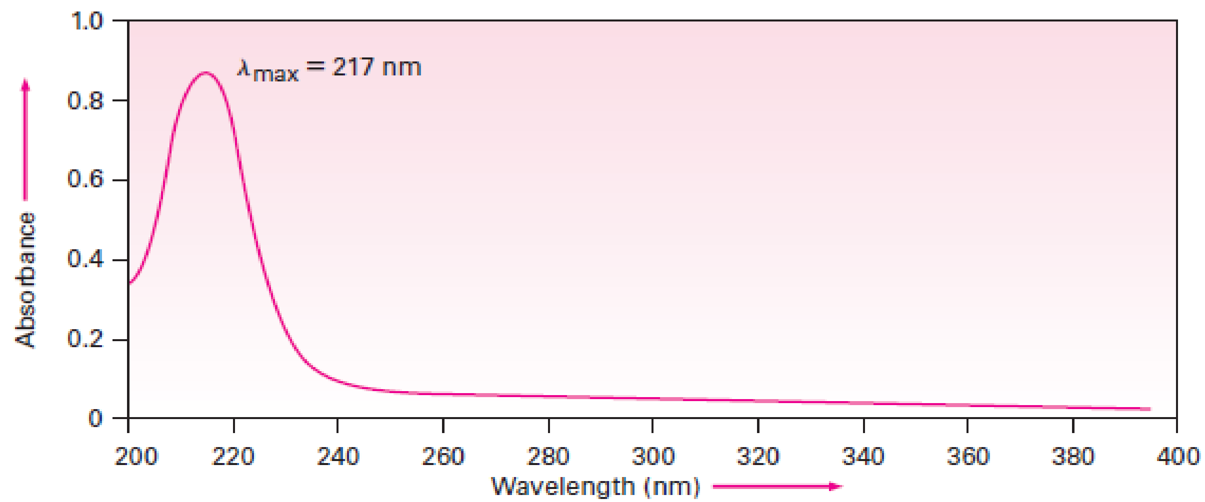
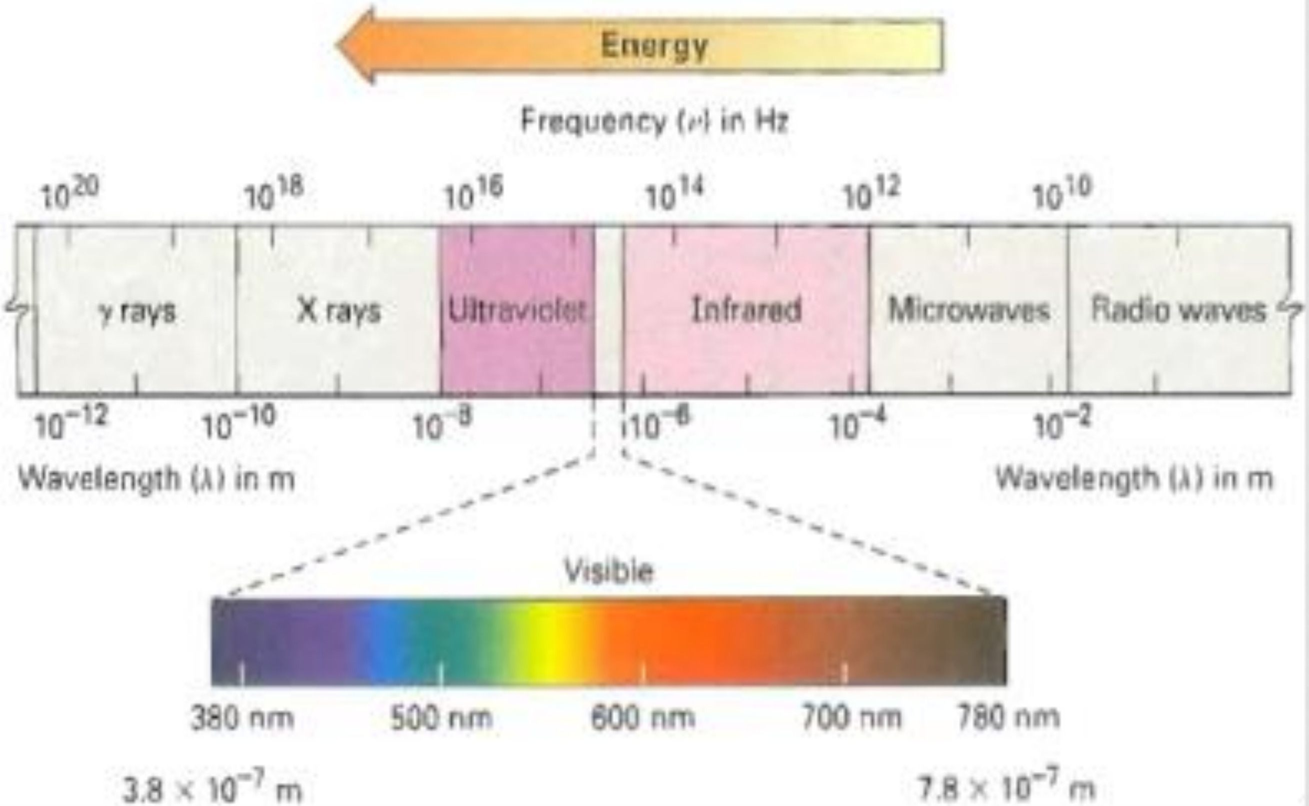


Figure 14.12 The ultraviolet spectrum of 1,3-butadiene, λ_{max} = 217 nm.

Figure 12.10 The electromagnetic spectrum covers a continuous range of wavelengths and frequencies, from radio waves at the low-frequency end to gamma (γ) rays at the high-frequency end. The familiar visible region accounts for only a small portion near the middle of the spectrum.

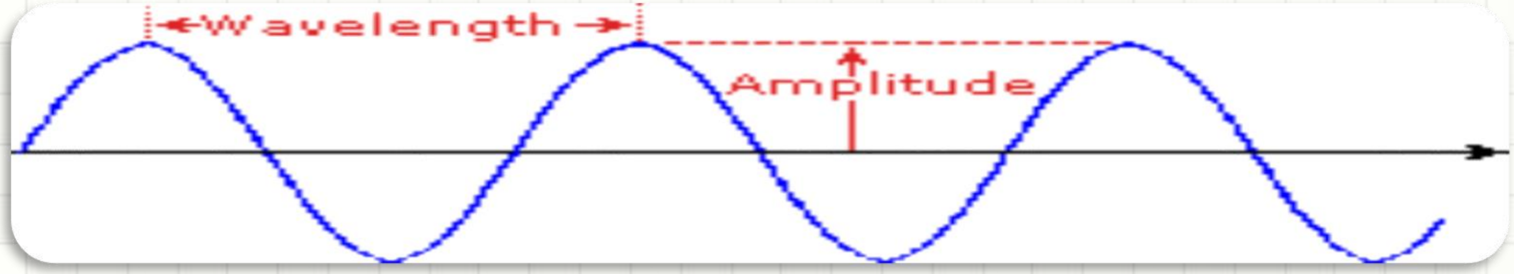


Electromagnetic radiation is often said to have dual behavior. In some respects, it has the properties of a particle, called a *photon,* yet in other respects it behaves as an energy wave.

Like all waves, electromagnetic radiation is characterized by:

- The wavelength, λ (Greek lambda), is the distance from one wave maximum to the next.
- The frequency, υ (Greek nu), is the number of waves that pass by a fixed point per unit time, usually given in reciprocal seconds (s^{-1}), or hertz, Hz (1 Hz = s^{-1}).
- The amplitude is the height of a wave, measured from midpoint to peak.

Electromagnetic Radiation



The various forms of electromagnetic radiation differ in their frequency and, therefore, their energy. The energy of electromagnetic radiation can be calculated in electron volts from the following equation:

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

$$c = \nu \lambda$$

- As photon is subjected to energy, so

$$\varepsilon = h\nu = \frac{hc}{\lambda}$$

where h = Planck's constant (6.62×10^{-34} J · s = 1.58×10^{-34} cal · s).

C= speed of the light ($3 * 10^{10}$) cm /second

V = the frequency (hertz), and λ is the wavelength (cm).

UV / Visible Spectroscopy

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.

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

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, …E_n, etc is called as electronic transition and the difference is as:

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

ΔE = 35 to 71 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.

Interaction of EMR with matter

3. Rotational Energy Levels:

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

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

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 95%, or hexane.

The useful information obtained from the UV _Visible spectrum of any compound are:

1. The wave length of maximum absorption λmax .
2. The intensity of absorption.

The compound should be dissolved in some suitable solvent that doesn't itself absorb light in the region under investigation.

The position of the absorption peaks of a compound may be shifted if different solvents are used.

However, the λmax for non polar compounds is generally the same in alcohol and hexane, while λmax for polar compounds is usually shifted dependent on the polarity of the solvent used

THEORY INVOLVED

- When a beam of light falls on a solution or homogenous media ,a portion of light is reflected ,from the surface of the media, a portion is absorbed within the medium and remaining is transmitted through the medium.
- Thus if I_0 is the intensity of radiation falling on the media
- I_r is the amount of radiations reflected,
- I_a is the amount of radiation absorbed &
- I_t the amount of radiation transmitted then

$$I_0 = I_r + I_a + I_t$$

- The absorbance A or optical density is given by:
- $A = \log I_0 / I$
- The range of absorbance commonly recorded is 0 to 2
- Transmittion is the ratio of the transmitted light to incident light: $T = I / I_0$
- $A = \log 1/T$