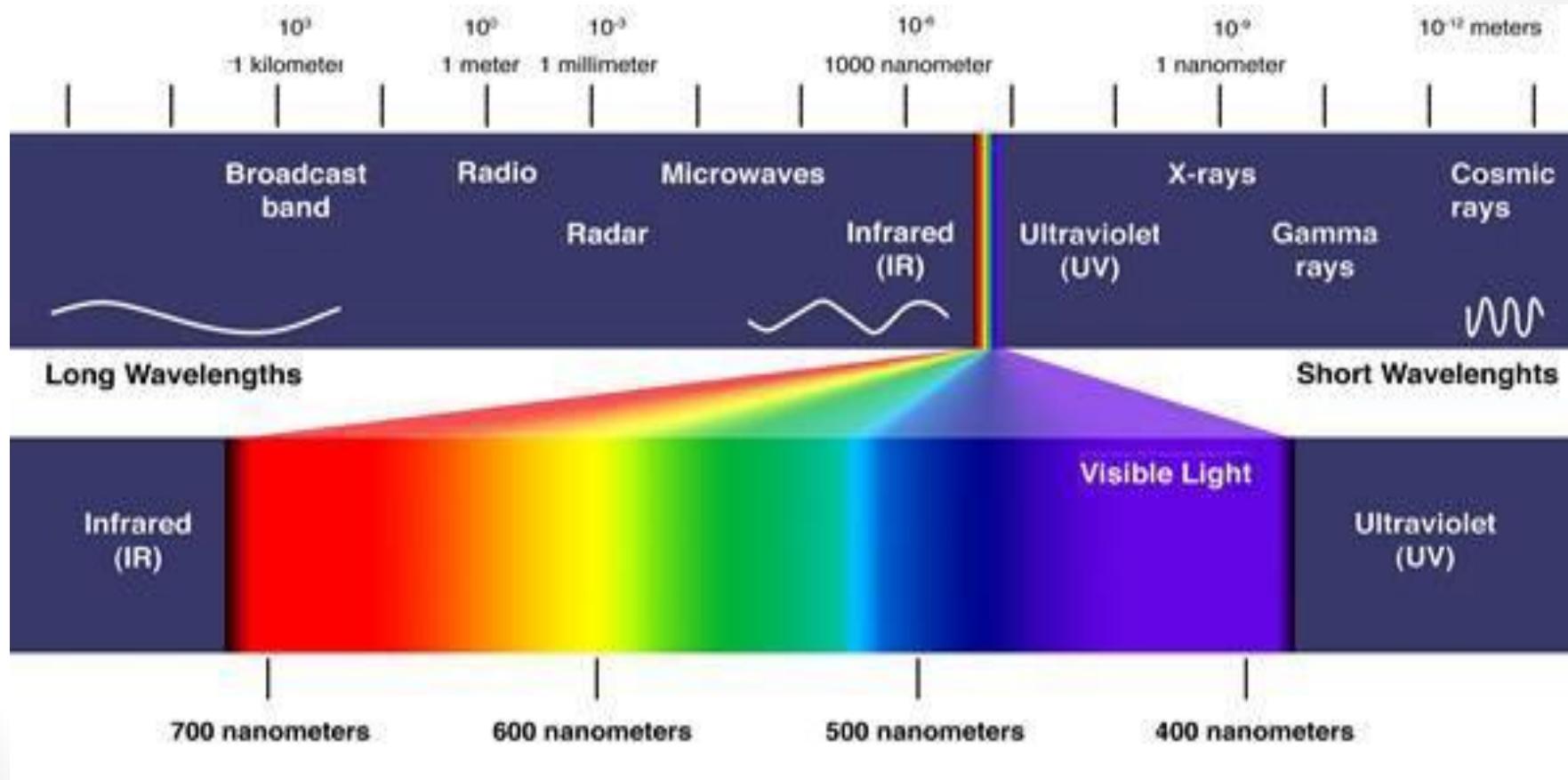


# SPECTROSCOPIC METHODS

## BASICS OF SPECTROSCOPY

### UV SPECTROSCOPY

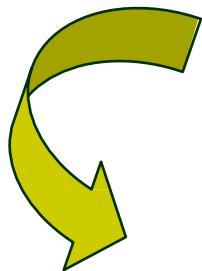


# WHAT IS SPECTROSCOPY?

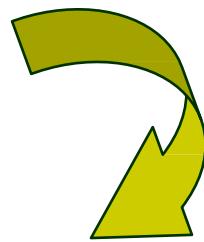
The study of the interaction between  
ELECTROMAGNETIC (EM) RADIATION and MATTER

# SPECTROSCOPIC ANALYSIS

covers



ATOMIC  
SPECTROSCOPY



MOLECULAR  
SPECTROSCOPY

Atomic spectroscopy means radiation interacts with atom in ground state.

Molecular spectroscopy means radiation interacts with molecules.

## **QUANTIZATION OF ENERGY**

It is the emission of energy in the form of quanta or discrete packets.

# Molecular spectroscopy

## Types of energies associated with molecules:

1. Electronic energy: associated with transition of an electron from ground state energy level to excited state energy level (molecular orbitals).
2. Vibrational energy: when the center of gravity does not change due to to and fro motion of the nuclei of the molecule , the molecule is said to possess vibrational energy.
3. Rotational energy: due to rotations of the molecule about its center of gravity in gas phase.
4. Translational energy: during motion if the center of gravity of the molecule changes the molecule possesses translational energy.

The total energy of the molecule is the sum of all these energies:

$$E = E_{el} + E_{vib} + E_{rot} + E_{tr}$$
$$E_{el} \gg E_{vib} \gg E_{rot} > E_{tr}$$

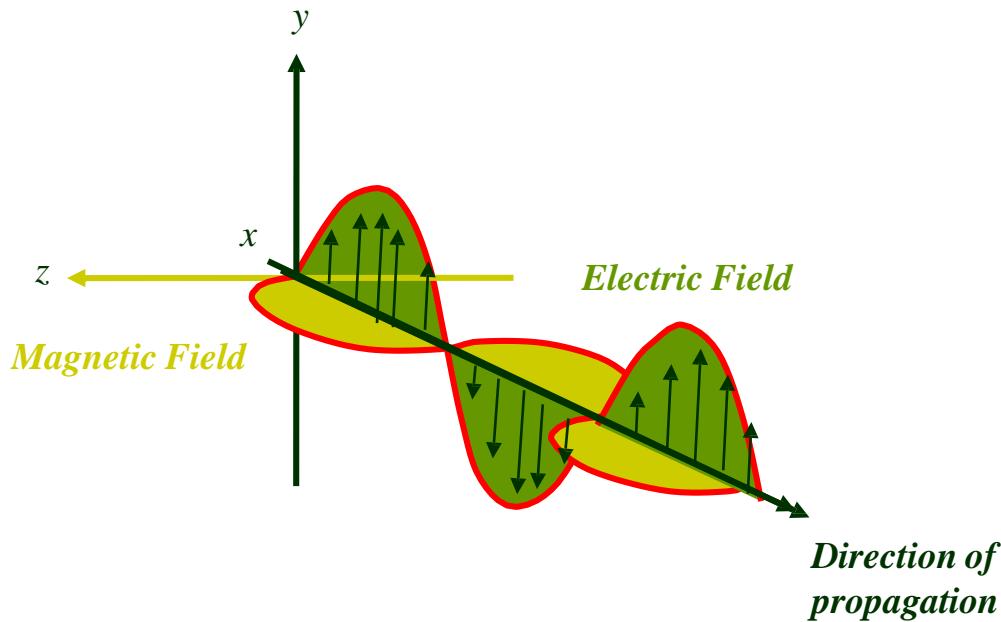
# To Understand Spectroscopy We Must Understand Electromagnetic Radiation

What is Electromagnetic Radiation?

- is a form of energy that has both Wave and Particle Properties.
- For example: Ultraviolet, visible, infrared, microwave, radio wave.

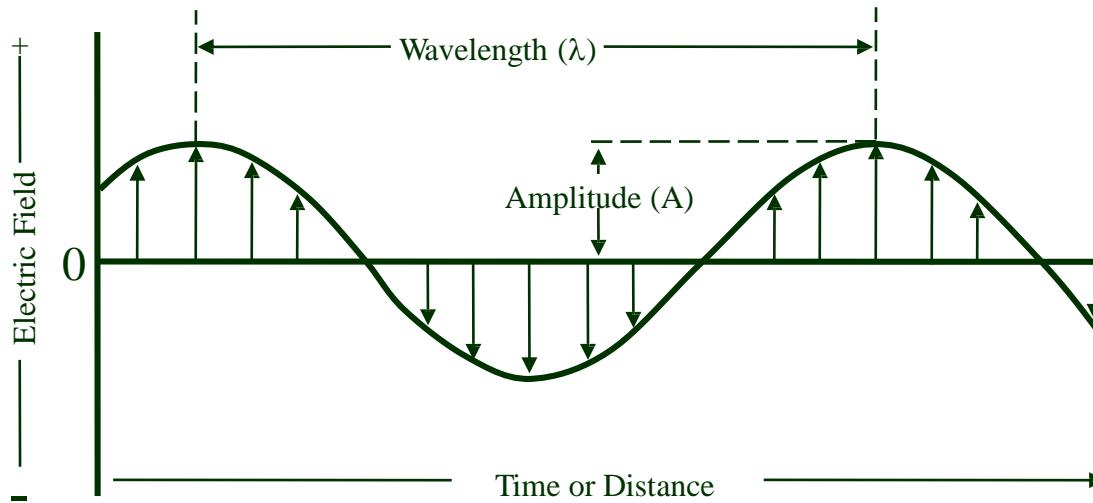
# WAVE PROPERTIES

- EM radiation is conveniently modeled as waves consisting of perpendicularly oscillating electric and magnetic fields, as shown below.



- At  $90^\circ$  to the direction of propagation is an oscillation in the **ELECTRIC FIELD**.
- At  $90^\circ$  to the direction of propagation and  $90^\circ$  from the electric field oscillation (orthogonal) is the **MAGNETIC FIELD** oscillation.

# Wave parameters



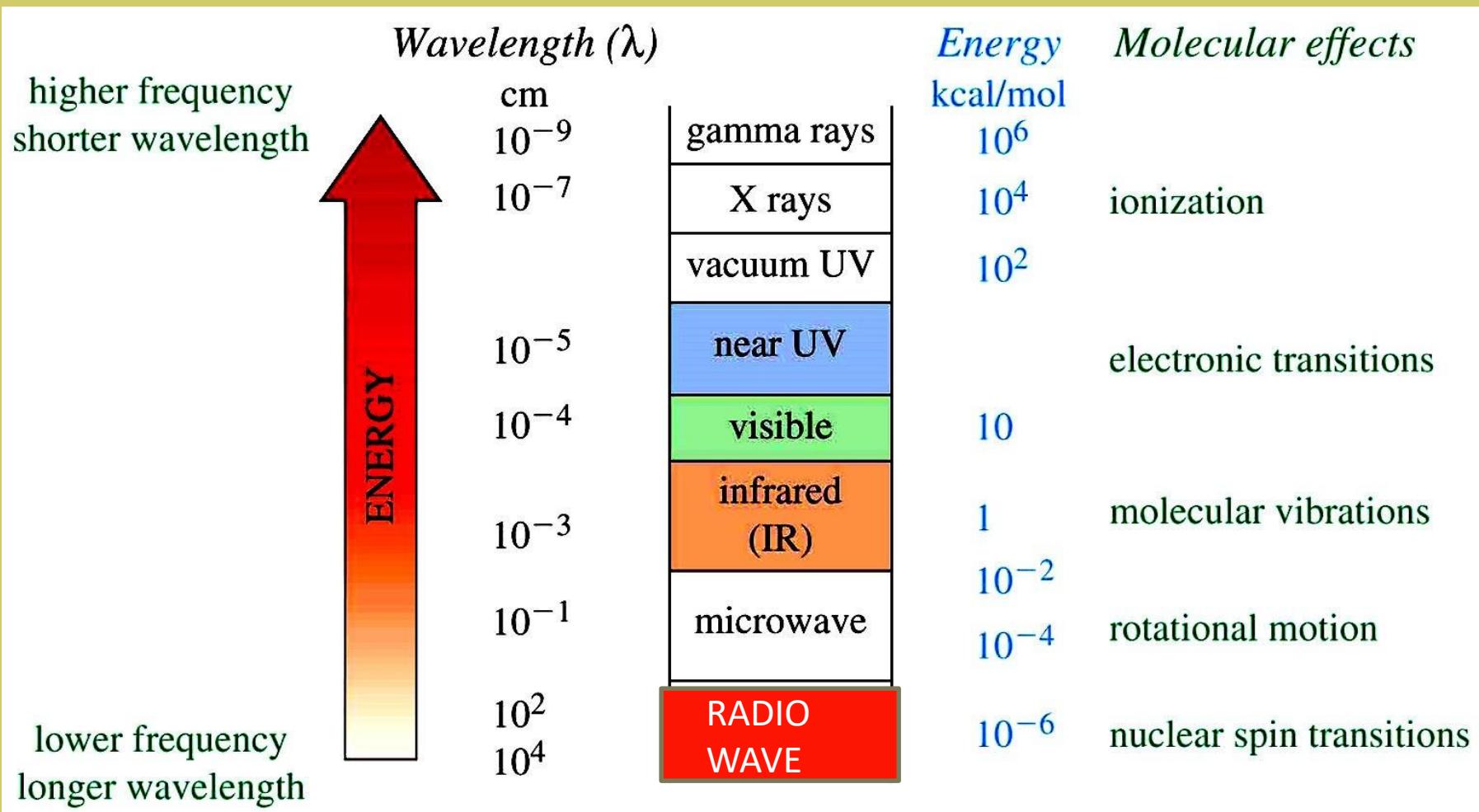
We Use Symbols to Designate the Various Properties of Waves

- $\lambda$  is the wavelength of the waves
- $\nu$  is the frequency of the waves
- $c$  is the speed of light

# Definitions:

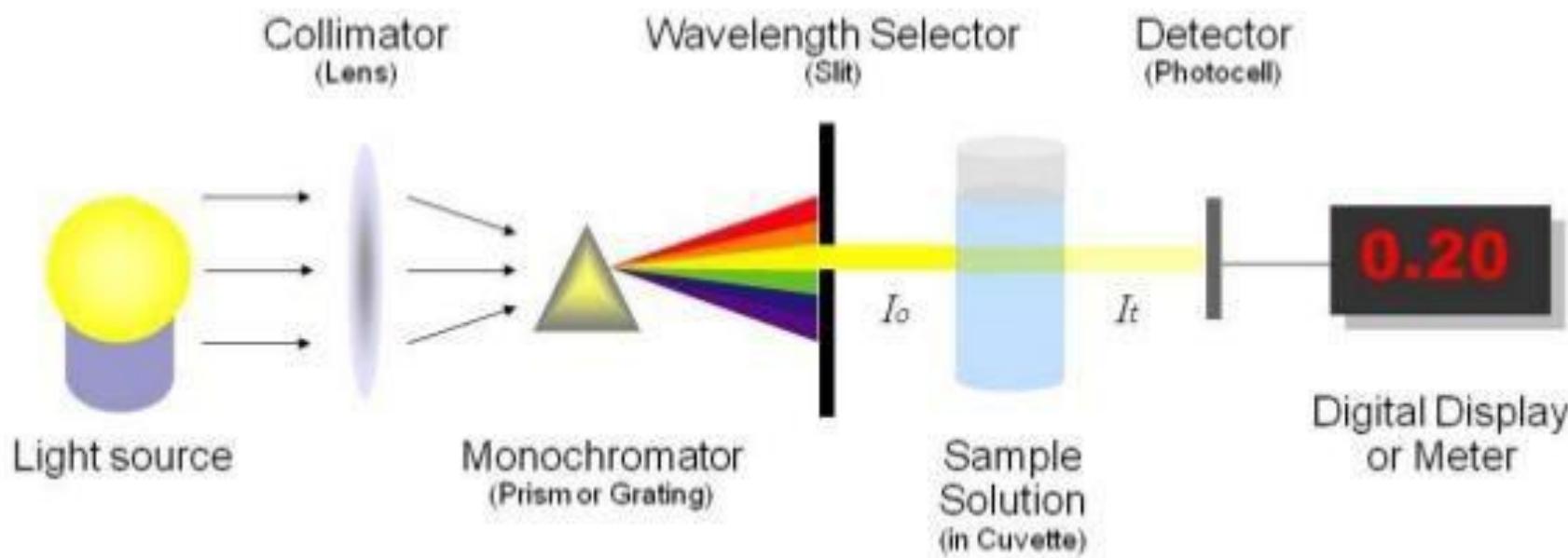
- **Period** ( $p$ ) – the time required for one cycle to pass a fixed point in space.
- **Frequency** ( $v$ ) – the number of cycles which pass a fixed point in space per second.
- **Amplitude** ( $A$ ) – The maximum length of the electric vector in the wave (Maximum height of a wave).
- **Wavelength** ( $\lambda$ ) – The distance between two identical adjacent points in a wave (usually maxima or minima).
- **Wavenumber** ( $\bar{v}$ ) - The number of waves per cm in units of  $\text{cm}^{-1}$ .

- **Purpose of each Electromagnetic Radiation**



# Spectrophotometer

To measure the amount of light that a sample absorbs



- **Visible spectrophotometer:** uses light over visible range (400 - 700 nm) of electromagnetic radiation spectrum.

## Absorption: The Beer-Lambert Law

August Beer (1825-1863): Added absorption co-efficient and related to conc. in solution.

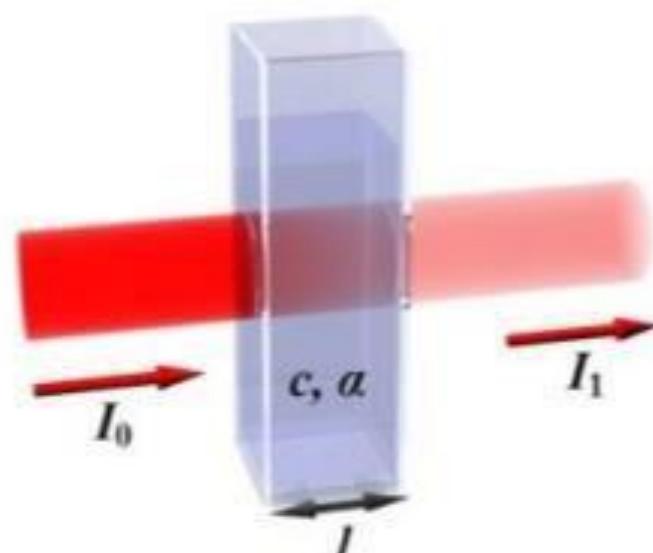
Pierre Bouguer

Astronomer: Light is diminished as it passes through the atmosphere.

Johan Lambert

Mathematician, first to prove that  $\pi$  is irrational.  
No absorption coefficient

$$A = -\log(I_1 / I_0) = \epsilon cl$$



$\epsilon$ : Extinction coefficient

$c$ : Concentration

$l$ : Path length

# UV Spectroscopy

The Beer-Lambert Law:  $A = \epsilon c l$

- for most UV spectrometers,  $l$  would remain constant (standard cells are typically 1 cm in path length)
- Concentration  $c$  is typically varied depending on the strength of absorption observed or expected – typically dilute – sub .001 M
- molar absorptivities  $\epsilon$  vary by orders of magnitude:
  - values of  $10^4$ - $10^6$  are termed **high intensity absorptions**
  - values of  $10^3$ - $10^4$  are termed **low intensity absorptions**
  - values of 0 to  $10^3$  are the absorptions of **forbidden transitions**

$A$  is unitless, so the units for  $\epsilon$  are  $\text{cm}^{-1} \times \text{M}^{-1}$  and are rarely expressed

Since path length and concentration effects can be easily factored out, absorbance simply becomes proportional to  $\epsilon$ , and the y-axis is expressed as  $\epsilon$  directly or as the logarithm of  $\epsilon$

# UV Spectroscopy

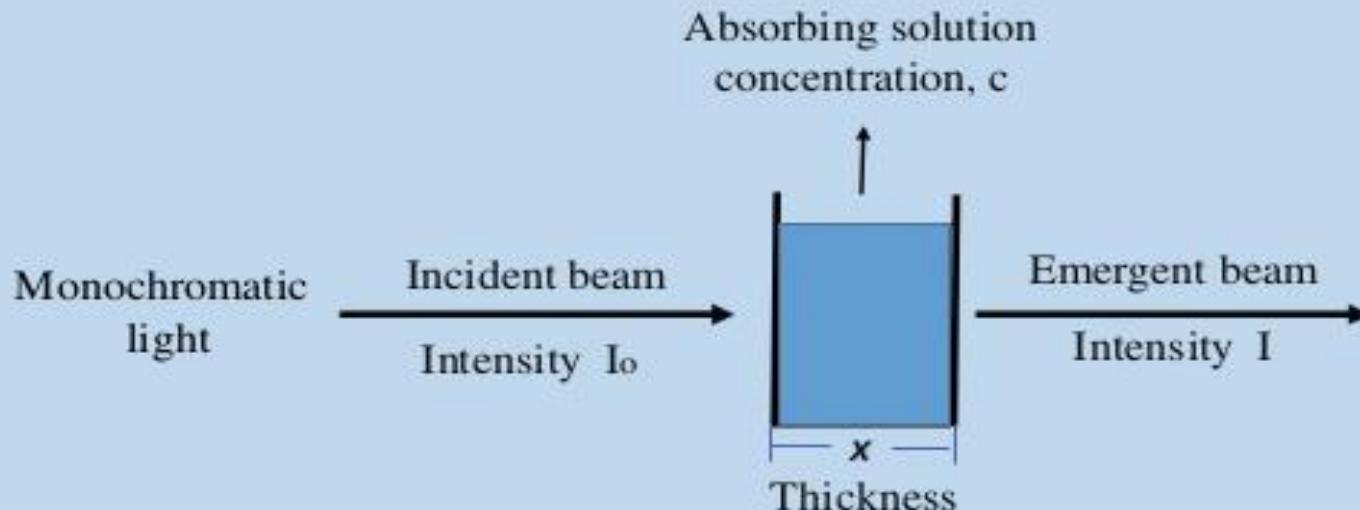
From an experimental point of view, three other considerations must be made:

- a longer **path length,  $l$** , through the sample will cause more UV light to be absorbed – linear effect
- the greater the **concentration,  $c$** , of the sample, the more UV light will be absorbed – linear effect
- some electronic transitions are more effective at the absorption of photon than others – **molar absorptivity,  $\epsilon$** ,  
*this may vary by orders of magnitude...*

# Derivation of Beer-Lambert's Law:

If material bodies are exposed to radiation, part of the incident radiation is absorbed, a part is scattered and a part is transmitted. As a result of absorption the intensity of light passing through material bodies, i.e. the intensity of transmitted light, decreases. The fraction of incident light absorbed depends on the thickness of the absorbing medium. Lambert derived a quantitative relationship between the decrease in intensity of a monochromatic light due to the passage through a homogeneous medium of thickness  $dx$  and the intensity of light  $I$ . This law is known as Lamber's law, and may be stated as

*The decrease in intensity of light with thickness of the absorbing medium at any point is directly proportional to the intensity of light.*



Mathematically it can be expressed as

$$-\frac{dI}{dx} \propto I \quad \dots \dots \dots \dots \dots \dots \dots \quad (1)$$

Where  $dI$  is a small decrease in intensity of light upon passing through a small distance  $dx$  and  $I$  is the intensity of the monochromatic light just before entering the medium. Equation (1) may be written as

$$-\frac{dI}{dx} = aI \quad \dots \dots \dots \dots \dots \dots \dots \quad (2)$$

Where -  $\frac{dI}{dx}$  is the rate of decrease of intensity with thickness  $dx$ ,  $a$  is called the *absorption co-efficient*. Integration of equation (2) after rearrangement gives,

$$- \ln I = ax + C \quad \dots \dots \dots \dots \dots \dots \quad (3)$$

Where C is a constant of integration. At  $x=0$ ,  $I=I_o$ . So,  $C = -\ln I_o$ . Introducing this in equation (3) we get,

Equation (4) can also be written as,

$$I = I_0 e^{-ax} \quad \dots \dots \dots \dots \dots \dots \quad (5)$$

Equation (5) can also be written as,

$$\log \frac{I}{I_0} = \frac{-a}{2.303} x \dots \dots \dots \quad (6)$$

$$or, \quad \log \frac{I}{I_0} = -a' x \quad \dots \dots \dots \dots \quad (7)$$

Where  $a' (= \frac{a}{2.303})$  is called *extinction co-efficient* and  $-\ln \frac{I}{I_o}$  is termed absorbance of the medium. Absorbance is represented by A.

Lambert's law was extended by beer who showed that when light passes through a solution of a given thickness the fraction of incident light absorbed is dependent not only on the intensity  $I$  of light but also on the concentration  $c$  of the solution. This is known as the Beer's law.

The two laws may be combined to write

$$-\frac{dI}{dx} \propto I \times c$$

Or

When the concentration,  $c$ , is expressed in  $\text{mol/L}$ ,  $b$  is called the molar absorption co-efficient.

As in the case of Lambert's law equation (9) may be transformed into,

$$\log \frac{I}{I_o} = \frac{-b}{2.303} \times c \times x \quad \dots \dots \dots \quad (10)$$

$$\log \frac{I}{I_o} = -\epsilon \times c \times x \quad \dots \dots \dots \quad (11)$$

Where  $\epsilon$  ( $= \frac{b}{2.303}$ ) is called the molar *extinction co-efficient* which is expressed in  $L/mol/cm$ . The molar *extinction co-efficient*  $\epsilon$  is dependent on the nature of the absorbing solute as well as on the wave length of the incident light used.

The expression (equation 11) is commonly known as Beer-Lambert's law.

### Transmittance (T) :

Experimental measurements are usually made in terms of transmittance (T), which is defined as:

$$T = I / I_o$$

where I is the light intensity after it passes through the sample and  $I_o$  is the initial light intensity. The relation between A and T is:

$$A = -\log T = -\log (I / I_o).$$

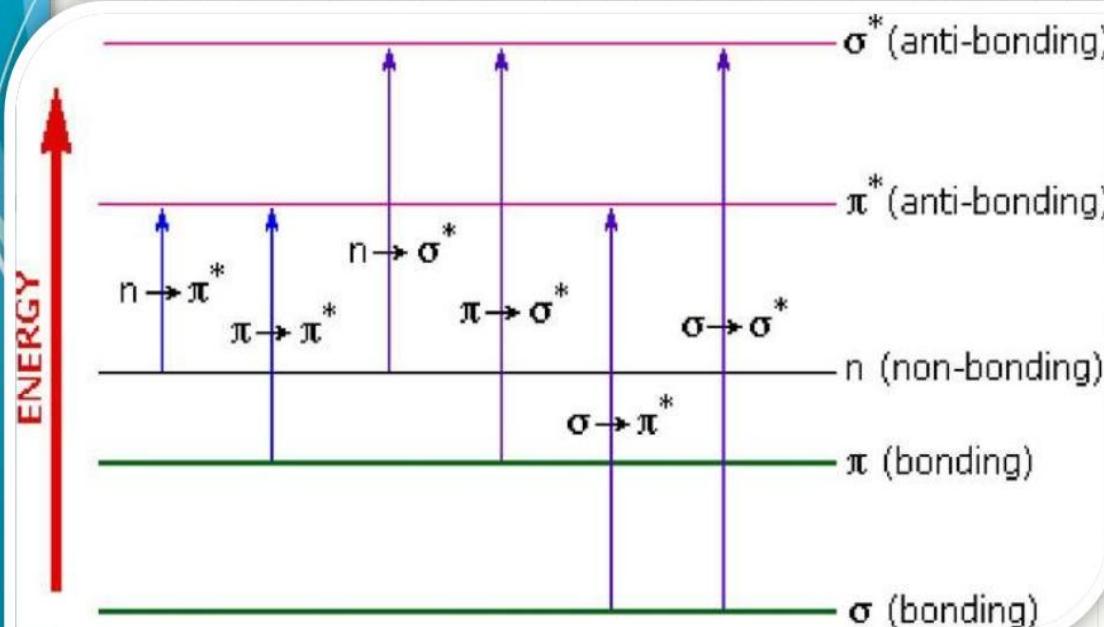
Transmittance is expressed as % T

## Limitations of the Beer-Lambert law:

The linearity of the Beer-Lambert law is limited by chemical and instrumental factors. Causes of nonlinearity include:

- 1) Deviations in absorptivity coefficients at ***high concentrations (>0.01M)*** due to electrostatic interactions between molecules in close proximity **scattering of light due to particulates** in the sample
- 2) **Fluorescence or phosphorescence** of the sample changes in refractive index at high analyte concentration shifts in chemical equilibria as a function of concentration.
- 3) Non-monochromatic radiation, deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band stray light.
- 4) Monochromatic beam of light should be applied.
- 5) Solute should not show association, dissociation in solution.
- 6) Solute and solvent should not show complex formation.
- 7) There should be no thermal equilibrium between ground state and excited state.

The possible electronic transitions can graphically shown as:



# ELECTRONIC TRANSITIONS

The absorption of uv and visible radiation corresponds to excitation of outer electrons. There are three types of electronic transitions that can be considered;

1. Transitions involving  $\pi$  ,  $\sigma$  and n electrons.
2. Involving charge transfer electrons and involving d and f electrons.
3. Most important are  $\sigma-\sigma^*$  transitions ,  $n-\sigma^*$  transitions &  $\pi-\sigma^*$  transitions.

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

- $\sigma$  electron from orbital is excited to corresponding anti-bonding orbital  $\sigma^*$ .
- The energy required is large for this transition.
- e.g. Methane ( $\text{CH}_4$ ) has C-H bond only and can undergo  $\sigma \rightarrow \sigma^*$  transition and shows absorbance maxima at 125 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).

5

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

&amp;

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

6

- 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.

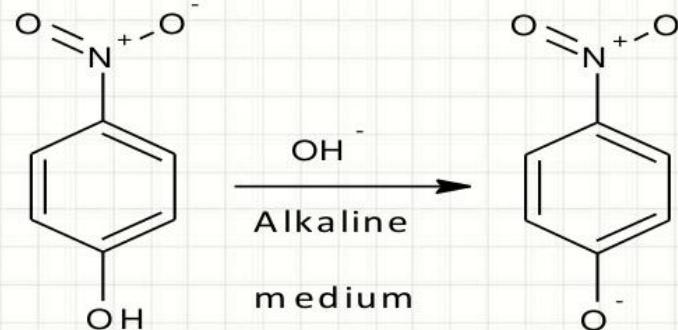
**1****• Bathochromic Shift (Red Shift)**

- When absorption maxima ( $\lambda_{\max}$ ) 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

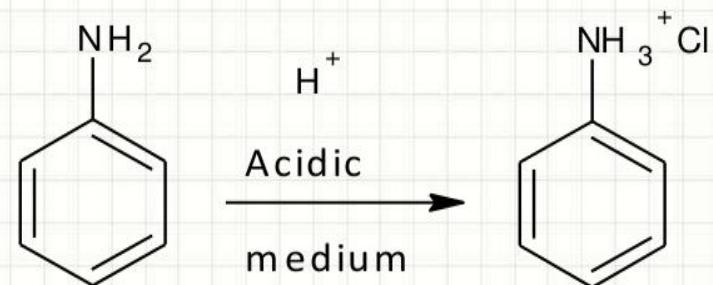
## • Hypsochromic Shift (Blue Shift)

- When absorption maxima ( $\lambda_{\max}$ ) 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

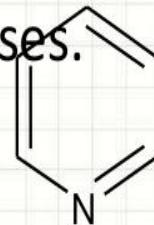
$$\lambda_{\max} = 280 \text{ nm}$$

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

## 3

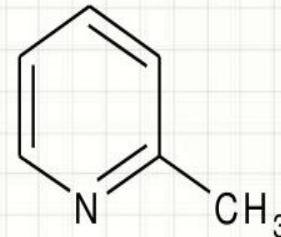
## • Hyperchromic Effect

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



Pyridine

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



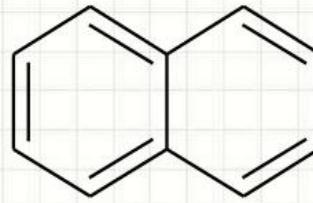
2-methyl pyridine

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

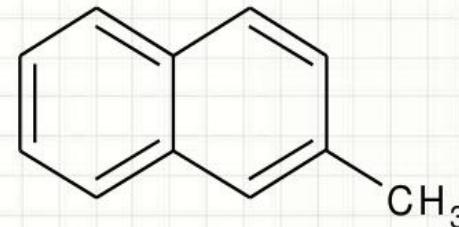
## 4

## • Hypochromic Effect

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

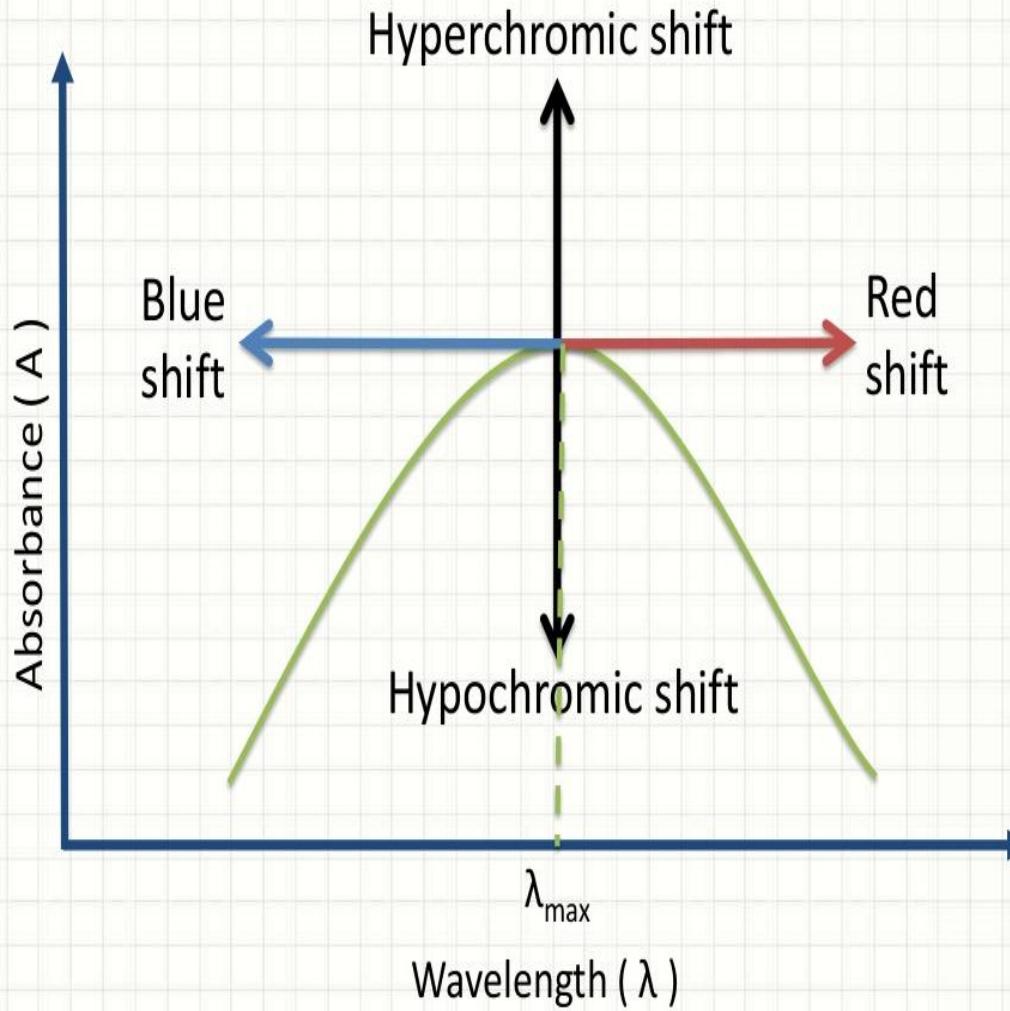


Naphthalene  
 $\epsilon = 19000$



2-methyl naphthalene  
 $\epsilon = 10250$

# Shifts and Effects



# CHROMOPHORE

- **Chromophore** – Any isolated covalently bonded group that shows a characteristic absorption in the UV/Visible region.

Eg: -C=C-, C = O

Any substance (groups) which absorbs radiation at particular wavelength this may or may not impart colour to the compound.

- **Chromophores types:**
  - The groups which contain a  $\Pi$  electrons and undergo  $\Pi$  to  $\Pi^*$  transitions
  - The groups which contain both  $\Pi$  and n electrons and undergo n to  $\Pi^*$  and  $\Pi$  to  $\Pi^*$  transitions.
- Compounds which posses  $\sigma$  to  $\sigma^*$  and n to  $\sigma^*$  transitions will show absorption in the vacuum UV region around 150nm and 190nm, so there wont be presence of any kind of chromophores within them.

# CHROMOPHORIC STRUCTURE

Group	Structure	nm
Carbonyl	>C=O	280
Azo	-N=N-	262
Nitro	-N=O	270
Thioketone	-C=S	330
Nitrite	-NO <sub>2</sub>	230
Conjugated Diene	-C=C-C=C-	233
Conjugated Triene	-C=C-C=C-C=C-	268
Conjugated Tetraene	-C=C-C=C-C=C-C=C-	315
Benzene		261

# AUXOCHROME

Clip slide

- **Auxochrome** is defined as any group, which does not itself act as a chromophore but whose presence brings about a shift of the absorption band towards the red end of the spectrum (longer wavelength)
- Chromophore + Auxochrome = newer chromophore
- ❖ Auxochrome is a colour enhancing group.
- ❖ The effect is due to its ability to extend the conjugation of a chromophore by sharing the nonbonding electrons.

- ❖ The new chromophore that is formed is of have a different value of absorption maximum as well as the extinction coefficient.

Benzene – 255nm ( $\epsilon_{\max}$  - 203)

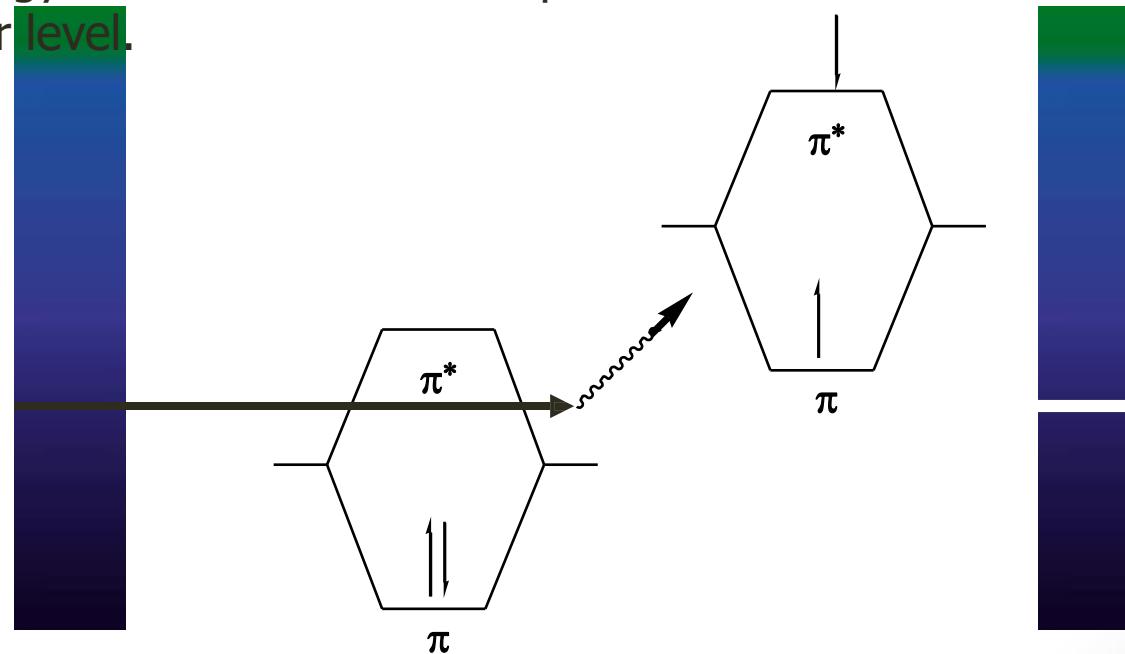
Aniline – 280nm ( $\epsilon_{\max}$  - 1430), so the auxochrome group is – NH<sub>2</sub>

Ex: - OH, - OR, - NH<sub>2</sub>, - NHR, - NR<sub>2</sub>, - SH etc.,

# UV Spectroscopy

## The Spectroscopic Process

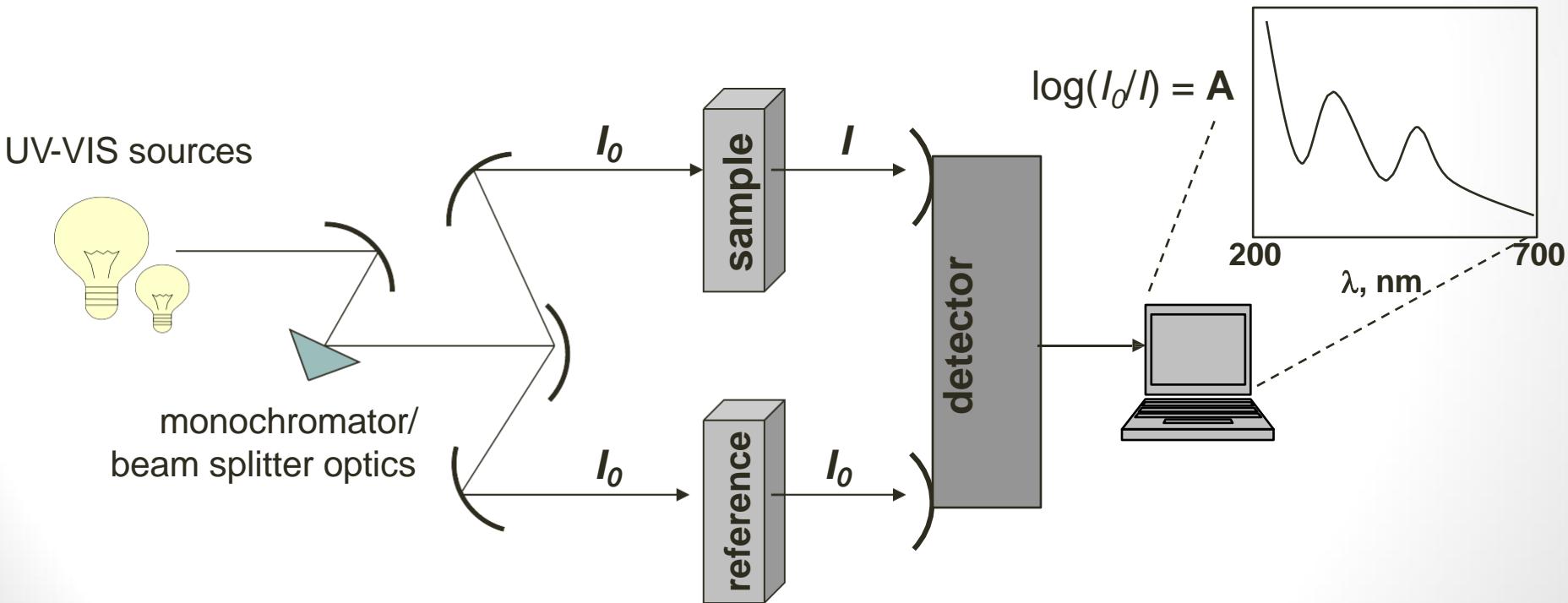
- In UV spectroscopy, the sample is irradiated with the broad spectrum of the UV radiation
- If a particular electronic transition matches the energy of a certain band of UV, it will be absorbed.
  - Energy promotes ground state electrons to higher state.
  - When uv light or visible light is passed through a compound with multiple bonds , a fraction of radiation absorbed & rest is emitted.
  - Energy of absorbed radiation promotes electron from lower to higher level.



# UV Spectroscopy

## Instrumentation and Spectra

- Here is a simple schematic that covers most modern UV spectrometers:



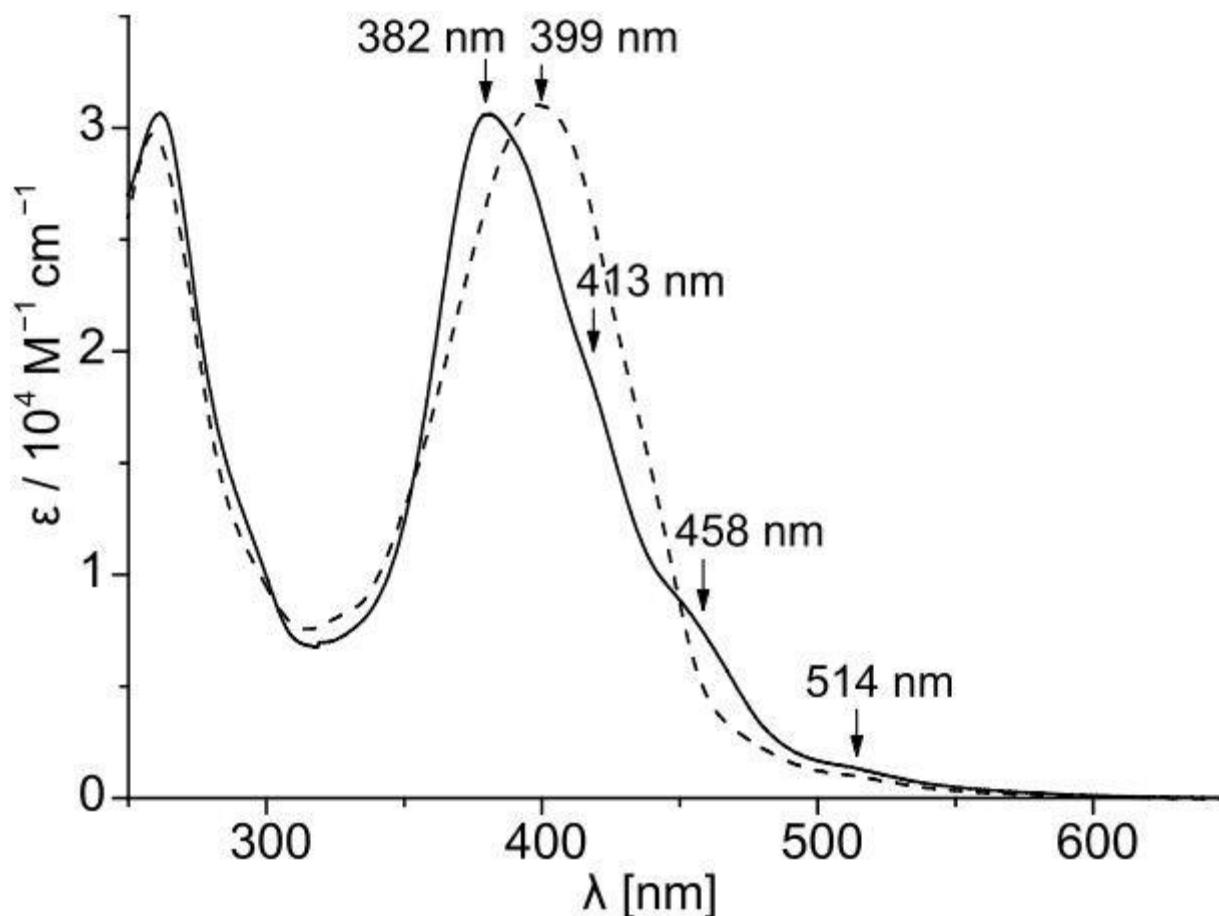
# UV Spectroscopy

## Instrumentation and Spectra,

- Two sources are required to scan the entire UV-VIS band:
  - Deuterium lamp – covers the UV – 200-330
  - Tungsten lamp – covers 330-700
- As with the dispersive IR, the lamps illuminate the entire band of UV or visible light; the monochromator (grating or prism) gradually changes the small bands of radiation sent to the beam splitter
- The beam splitter sends a separate band to a cell containing the sample solution and a reference solution
- The detector measures the difference between the transmitted light through the sample ( $I$ ) vs. the incident light ( $I_0$ ) and sends this information to the recorder

# UV Spectroscopy

- Due to the lack of any fine structure, spectra are rarely shown in their raw form, rather, the peak maxima are simply reported as a numerical list of “lambda max” values or  $\lambda_{\max}$



## APPLICATIONS OF U.V. SPECTROSCOPY:

### 1. Detection of Impurities

- o UV absorption spectroscopy is one of the best methods for determination of impurities in organic molecules. Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material. By also measuring the absorbance at specific wavelength, the impurities can be detected.

## 2. Structure elucidation of organic compounds.

- UV spectroscopy is useful in the structure elucidation of organic molecules, the presence or absence of unsaturation, the presence of hetero atoms.
  
- From the location of peaks and combination of peaks, it can be concluded that whether the compound is saturated or unsaturated, hetero atoms are present or not etc.

### 3. QUANTITATIVE ANALYSIS

- UV absorption spectroscopy can be used for the quantitative determination of compounds that absorb UV radiation. This determination is based on Beer's law which is as follows.

$$A = \log I_0 / I_t = \log 1/T = -\log T = abc = \epsilon bc$$

Where :

$\epsilon$  -is extinction co-efficient,

c- is concentration, and

b- is the length of the cell that is used in UV spectrophotometer.

## 4. QUALITATIVE ANALYSIS

- UV absorption spectroscopy can characterize those types of compounds which absorbs UV radiation. Identification is done by comparing the absorption spectrum with the spectra of known compounds.



## 6. DETECTION OF FUNCTIONAL GROUPS

- This technique is used to detect the presence or absence of functional group in the compound
  
- Absence of a band at particular wavelength regarded as an evidence for absence of particular group