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Presentation

OPTICAL TECHNIQUES PART-1

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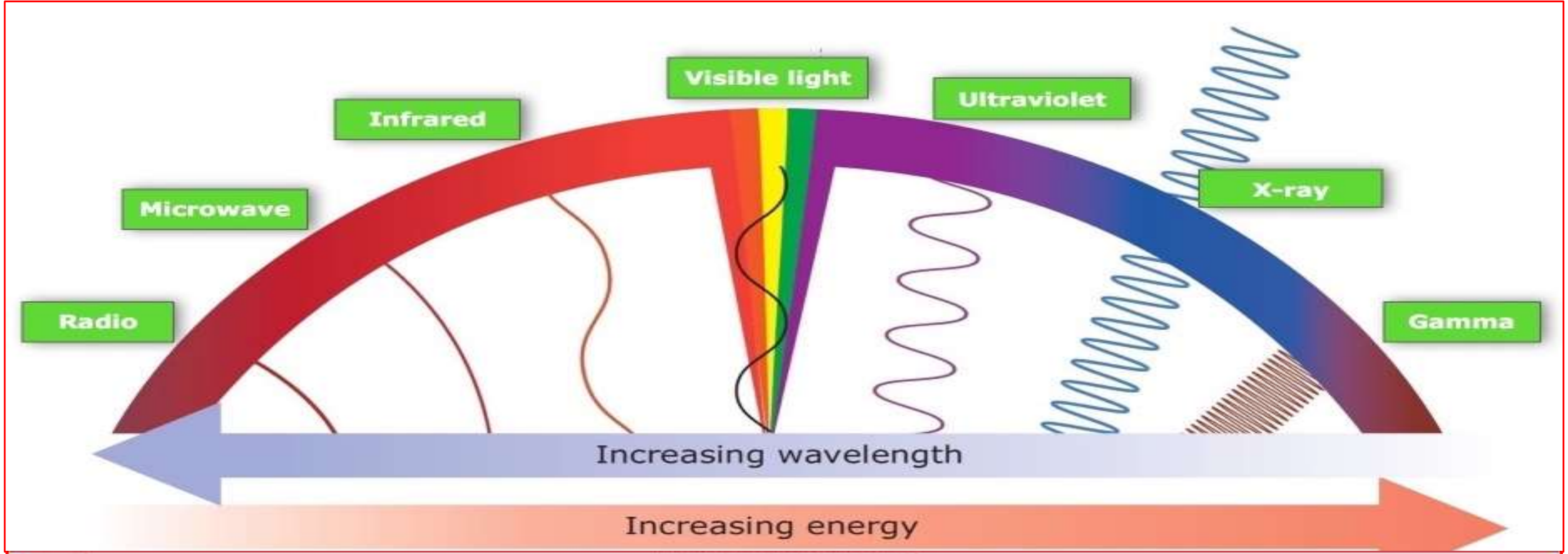
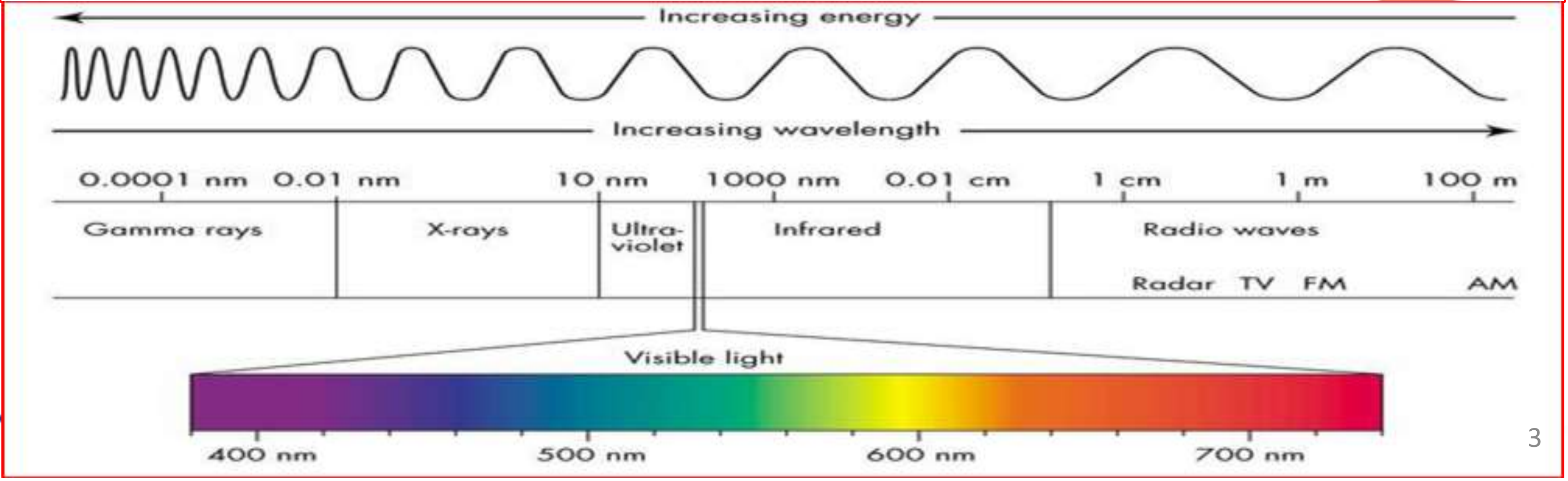
Optical Techniques

- ❑ Introduction
- ❑ Electromagnetic Spectrum
- ❑ Beer-Lambert Law
  - ❑ Calculate the concentration of a solution
  - ❑ Nucleic acid calculation
- ❑ Applications
  - ❑ Photometry
  - ❑ Spectrophotometer
  - ❑ Flame Emission Spectrophotometry
  - ❑ Atomic Absorption Spectrophotometry
  - ❑ Fluorometry
  - ❑ Luminometry

The Electromagnetic Spectrum

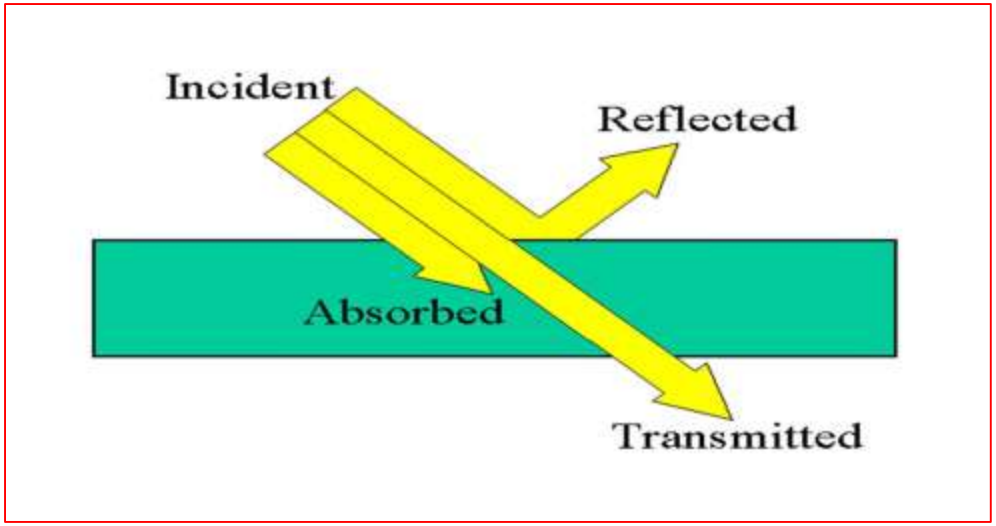




Optical Technique

The EM Radiation interact with biomolecule

- ❑ Reflected
- ❑ Absorbed
- ❑ Scattered
- ❑ Emitted
- ❑ Transmitted

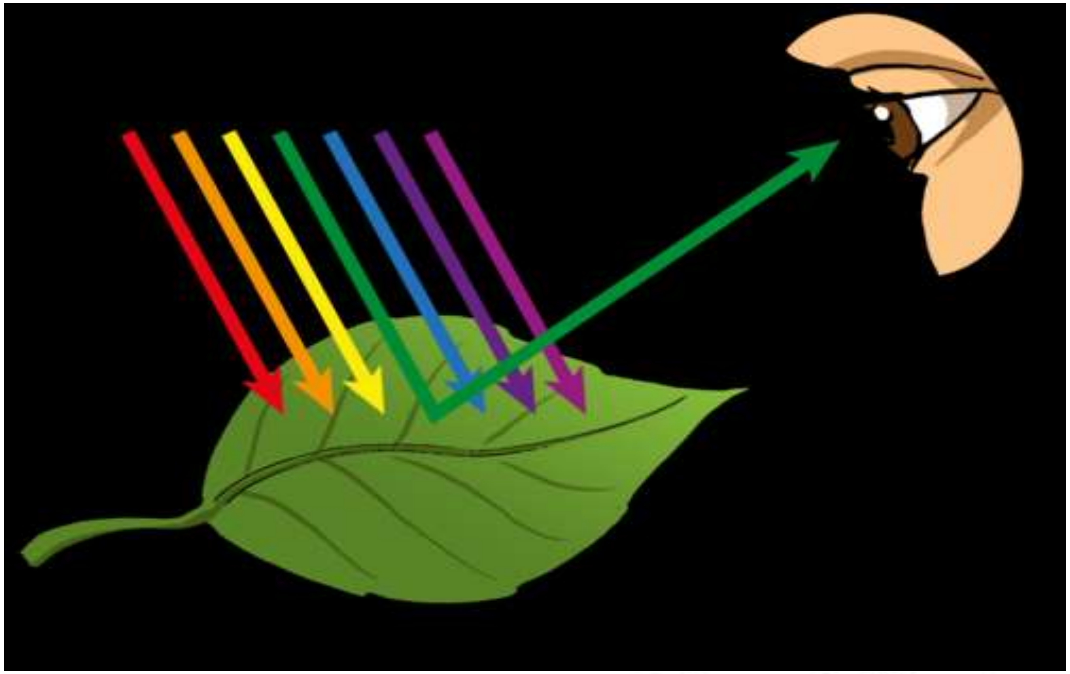


Optical Technique

Example:

The EM Radiation interact with biomolecule

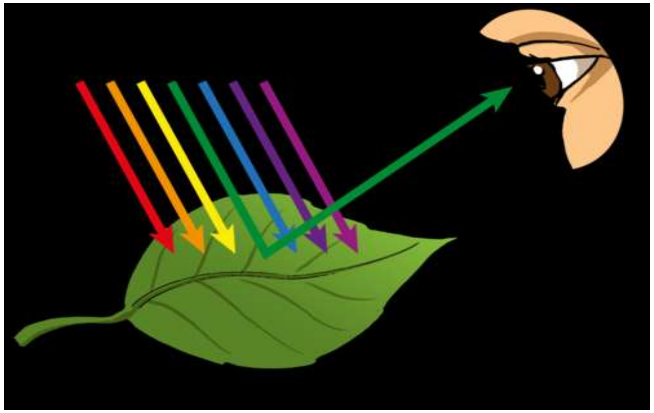
- Reflected



Absorption:

Examples:

- ❑Spectrophotometry
- ❑ Photometry
- ❑ X-ray spectroscopy

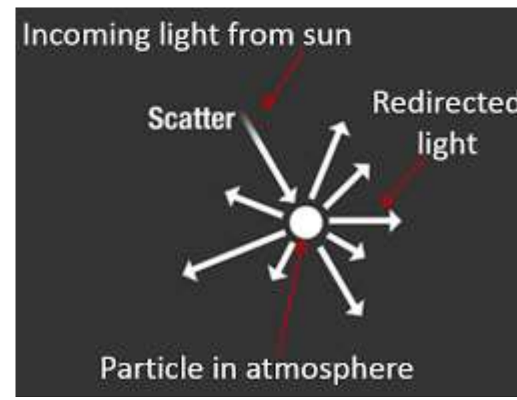


Scattering

Turbidity:

- The cloudiness of a solution caused by suspended particles that scatter light

- light scattered related to the concentration and sizes and shapes of the particles.

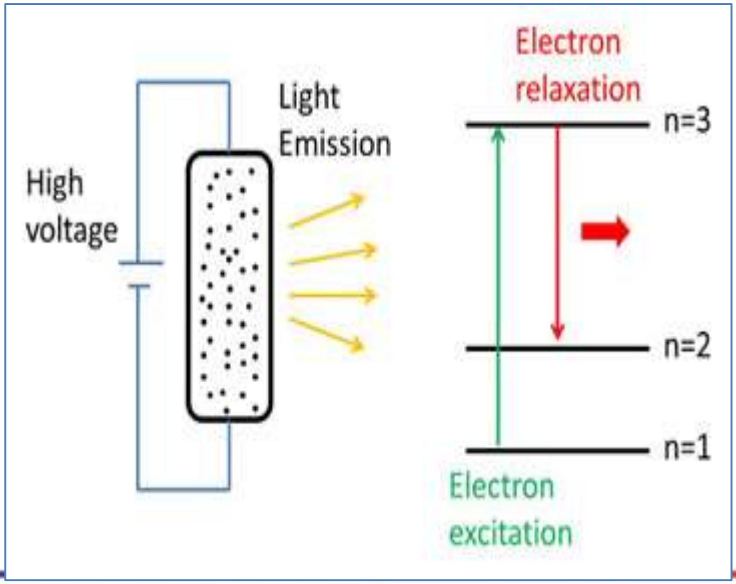


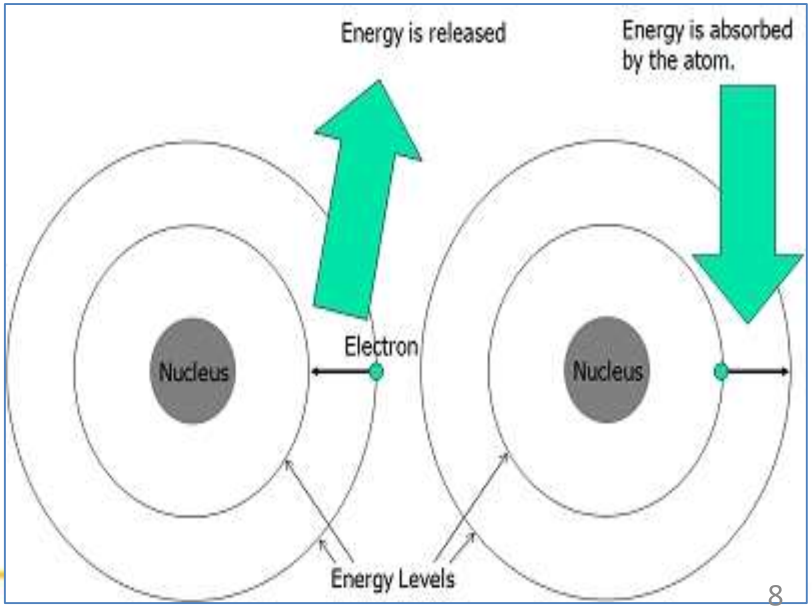
Turbidimetry:

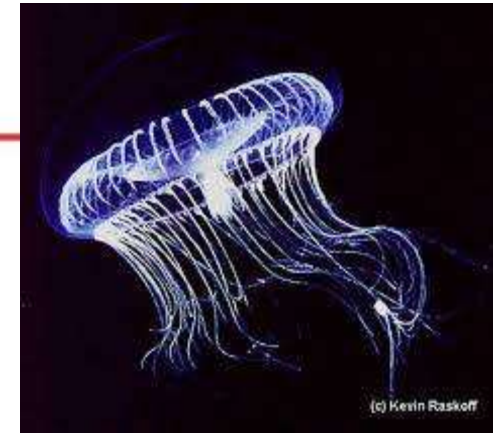
Performed through use of an instrument (spectrophotometer) measures the ratio of the intensity of the light transmitted through dispersion to the intensity of the incident light.

Emission

- When the atoms move to a lower energy state.
- Release of energy, usually in the form of a photon, which is a tiny energy packet.
- This energy emits from the atom in the form of radiation, generally away from the atom.







Examples of Emission:

Luminescence: the emission of light or radiant energy when an electron returns from an excited or higher energy level to a lower energy level.

Example:

Fluorescence

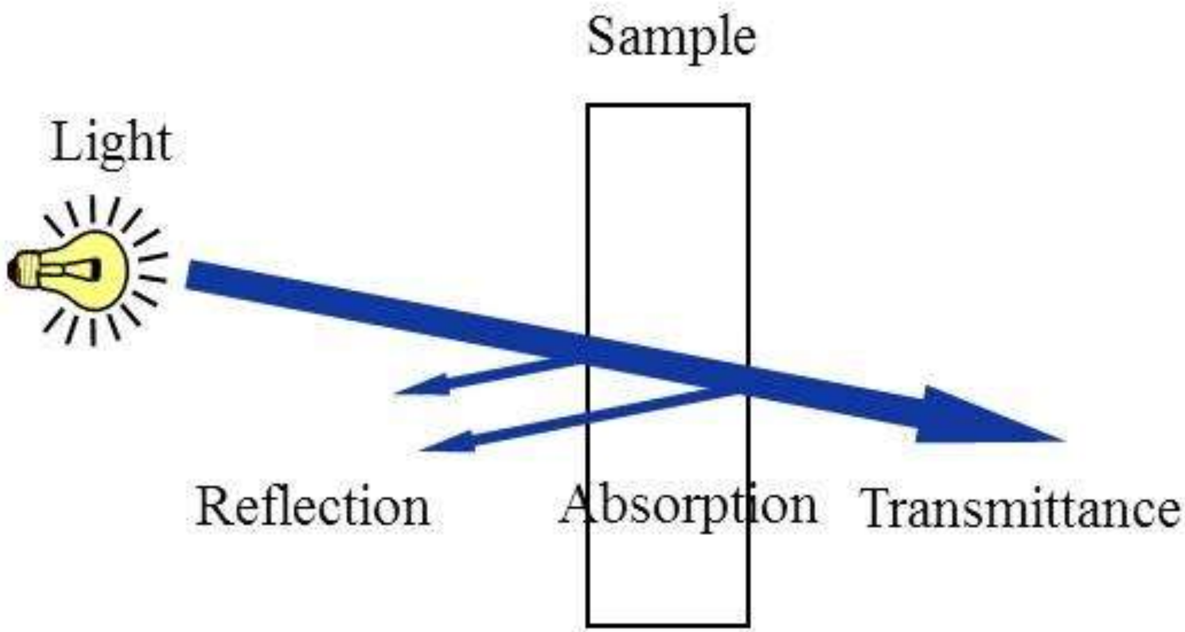
Phosphorescence

Chemiluminescence:

The emission of light by molecules in excited states produced by a chemical reaction,

Example: Fireflies

Relationship Between Transmittance and Absorbance



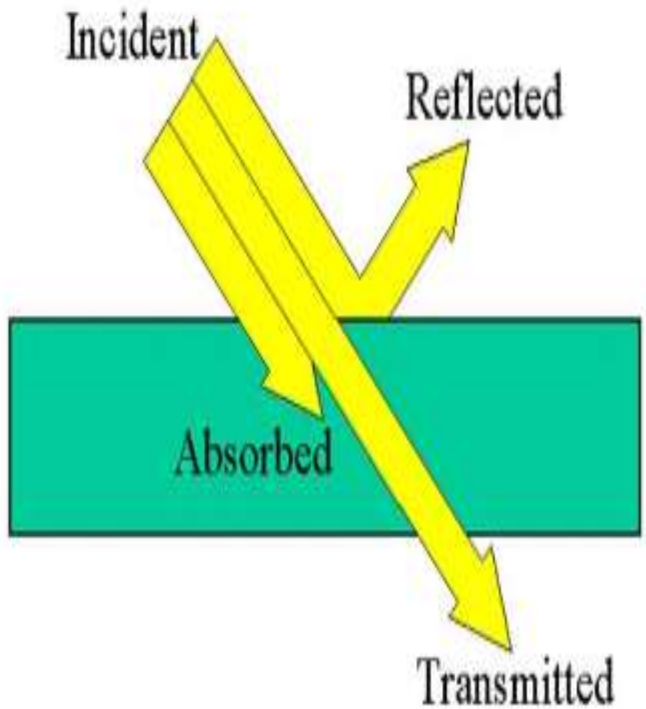
Reflection + Absorption + Transmittance = 100%

Relationship Between Transmittance and Absorbance

$I_0 = I_T + I_A + I_R$

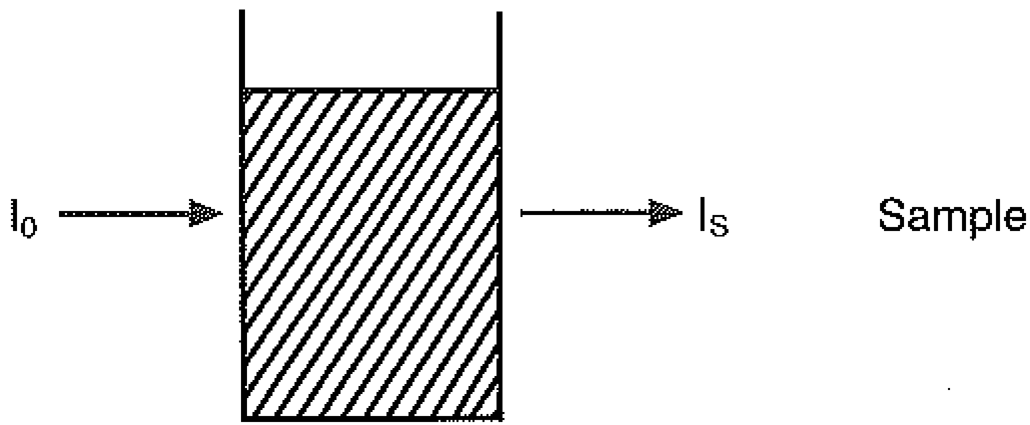
$T = I_T / I_0$	Transmissivity
$A = I_A / I_0$	Absorptivity
$R = I_R / I_0$	Reflectivity
$T \sim 1$:	Transparent
$T \sim 0$:	Opaque

$T + A + R = 1$



Relationship Between Transmittance and Absorbance

When an incident light beam with intensity ***I0***. passes through a square cell containing a solution of a compound that absorbs light of a specific wavelength, λ



- The intensity of the transmitted light beam ***Is*** is **less** than ***Io***,
- The transmitted light (T) is defined as

$$T = \frac{I_S}{I_0} \qquad (1)$$

Relationship Between Transmittance and Absorbance

❖ Some of the incident light may be **reflected** by

- Surface of the cell
- Absorbed by the cell wall or Solvent

❖ These factors are eliminated by **Reference cell**

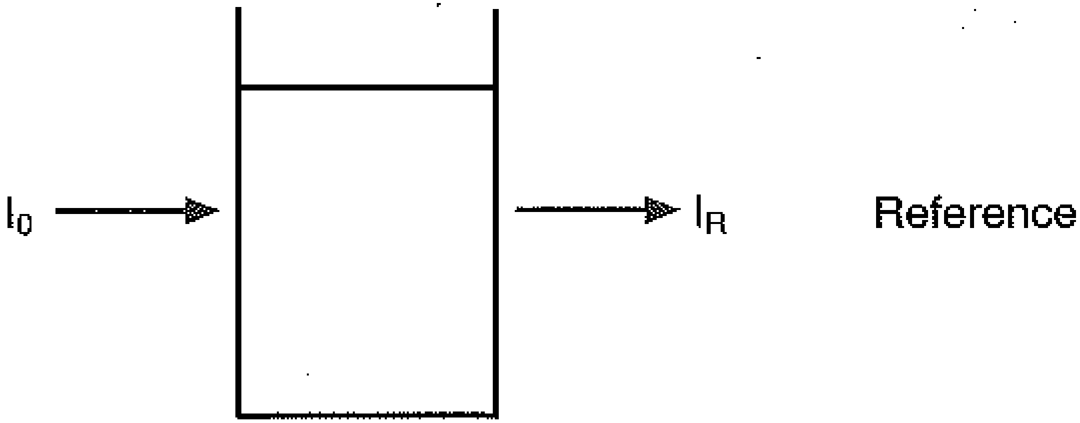
Reference Cell:

•Identical to the sample cell

•Except that the compound of interest (sample) is omitted from the solvent in the reference cell.

Transmittance

The Transmittance (T) through this Reference cell is

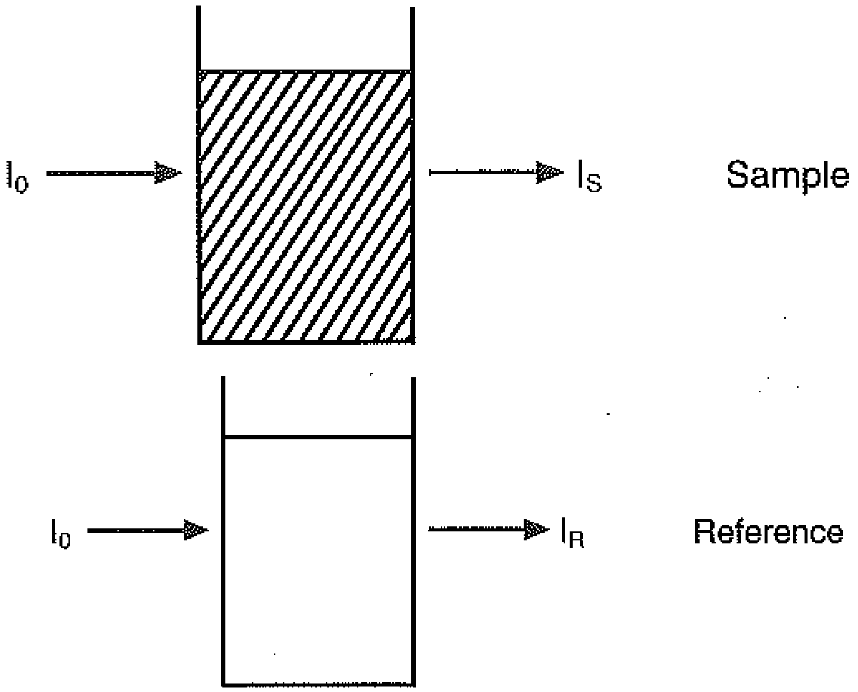


$$T = \frac{I_R}{I_O}$$

Light Transmittance of sample versus reference = Is / IR.

- Transmittance for the compound in solution is defined as

$$\boxed{T = \frac{I_s}{I_R}}$$



Relationship Between Transmittance and Absorbance

- In practice the Reference cell is inserted and the instrument adjusted to an arbitrary scale reading of 100 (100% T)

- Non-Absorbing samples: Transparent

- *Io = I;*
- *Io is incidence light and I is transmitted light*

- *T= 100%*

Transmittance

- Opaque samples
- *$I_o = 0$;*
- *Io is incidence light*
- *T= 0%*

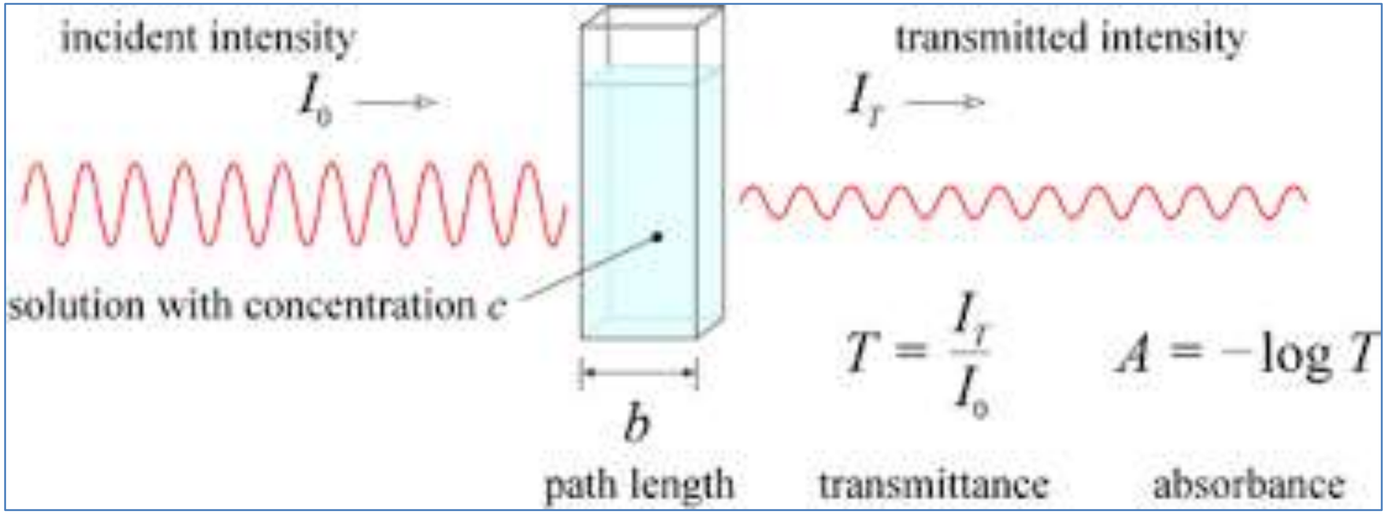
Absorbance (A)

Absorbance (A)

- The capacity of a substance to absorb radiation

- Molar absorptivity (Ɛ): A constant for a one molar solution of a given compound at a given **wavelength** and a **1cm path length** under prescribed condition of **solvent, temperature and ph**.

- Radiant Energy :



Relationship Between Transmittance (T) and Absorbance (A)

- Expressed as the logarithm (log) of the reciprocal of transmittance (T)

- The amount of light absorbed (A) as the incident light passes through the sample is equivalent to

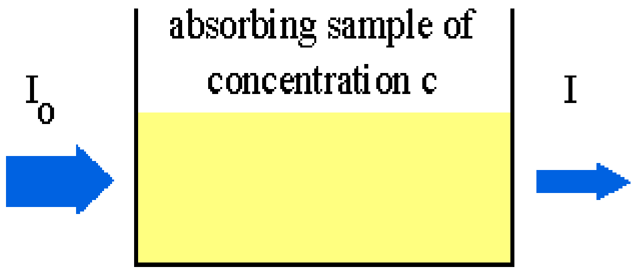
$$\text{Abs (A)} = \log (1/T)$$
$$= -\log T$$
$$= -\log \frac{I_S}{I_R}$$

$$T = \frac{I_S}{I_R}$$

Beer's Law

Absorbance is directly proportional to concentration of solution in which light has to passed.

$$\boxed{Abs = \varepsilon \ c}$$



ε = Molar extinction co-efficient
C= concentration of solution

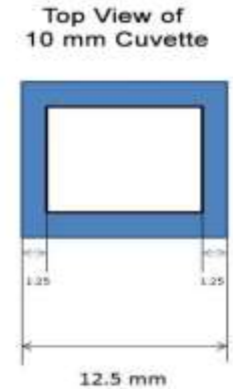
Lambert's Law

"The loss of light intensity when it propagates in a medium is directly proportional to intensity and path length."

Absorbance is proportional to **length of sample in which light passed**

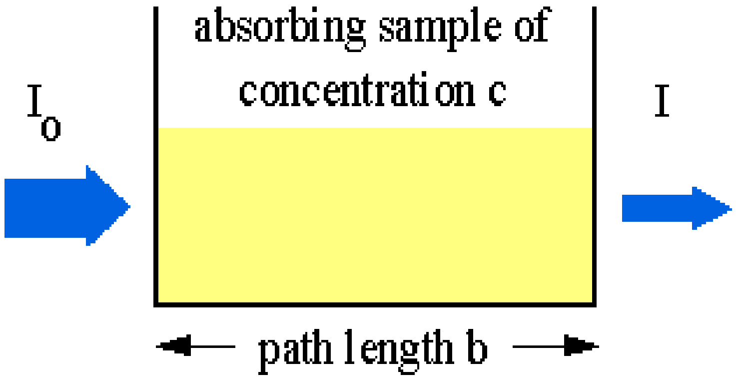
$$\text{Abs} = \log_{10} (1/T) \; \alpha \; l$$

L = Path length



$$\text{Abs} = \varepsilon \; l$$

ε = Molar extinction co-efficient



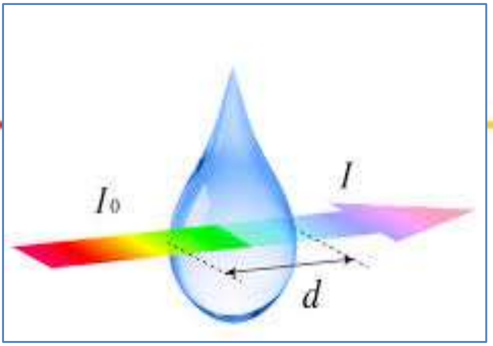
Beer-Lambert Law

Combining both

$$Abs = \log_{10} (1/T) \propto c\, l$$

$$Abs = \varepsilon\, c\, l$$

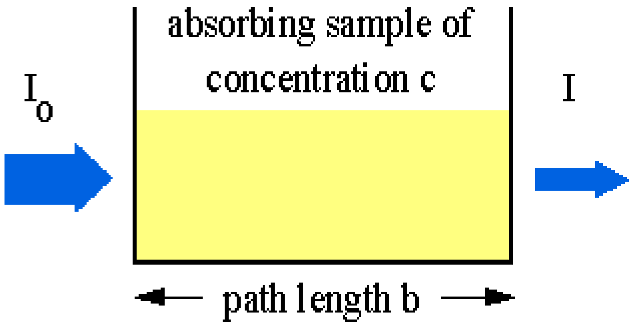
Beer-Lambert Law



Mathematically, Beer's law is expressed as

$$A = \boldsymbol{\varepsilon} \times l \times c$$

$$A = abc$$



A = Absorbance = the amount of light absorbed by the sample for a given wavelength

a = **ε** is the **molar absorptivity**

b = the distance that the light travels through the solution,

Light path in centimeters

c = Concentration of the absorbing compound (grams per liter)

Application

Beer-Lamberts law

Application

Generally, it can be used to determine

- concentrations of a particular substance,
- determine the molar absorptivity of a substance.

Spectrophotometry

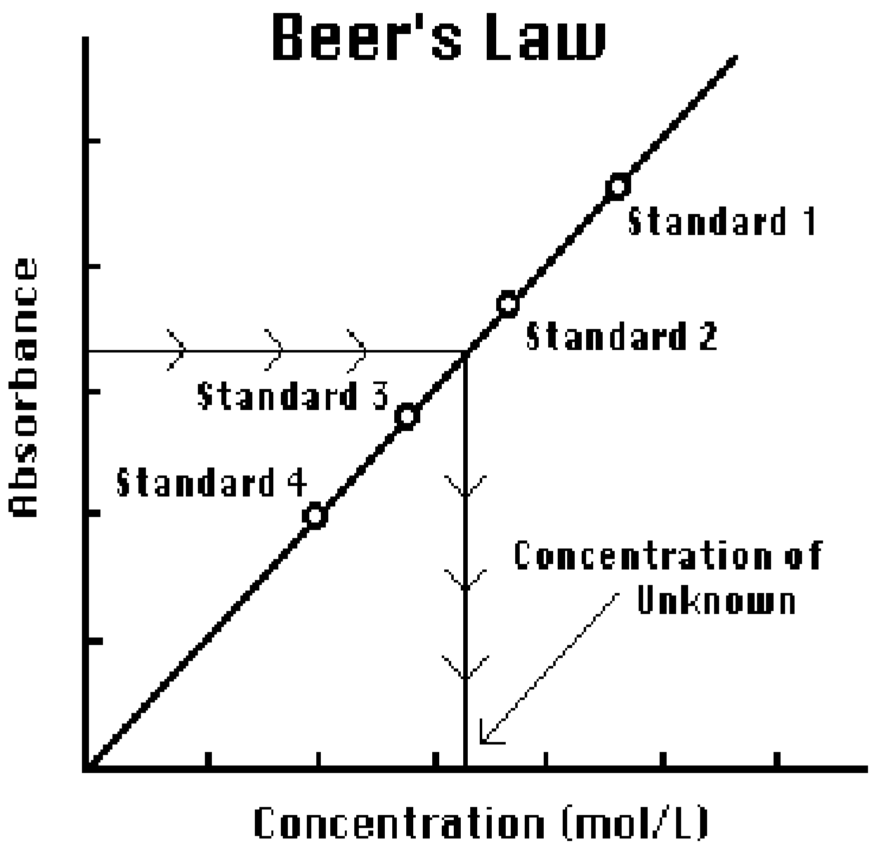
Example:

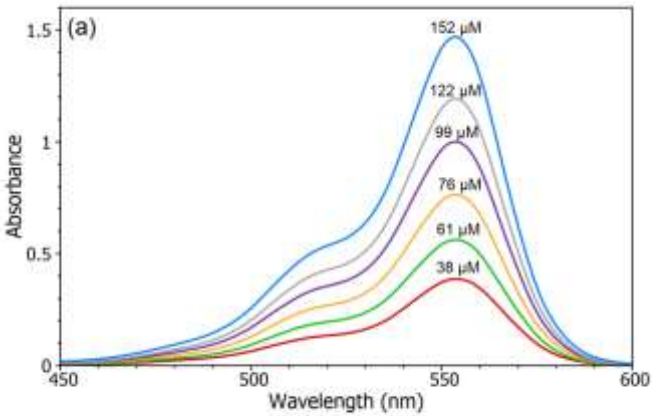
Determination of bilirubin in blood plasma samples.

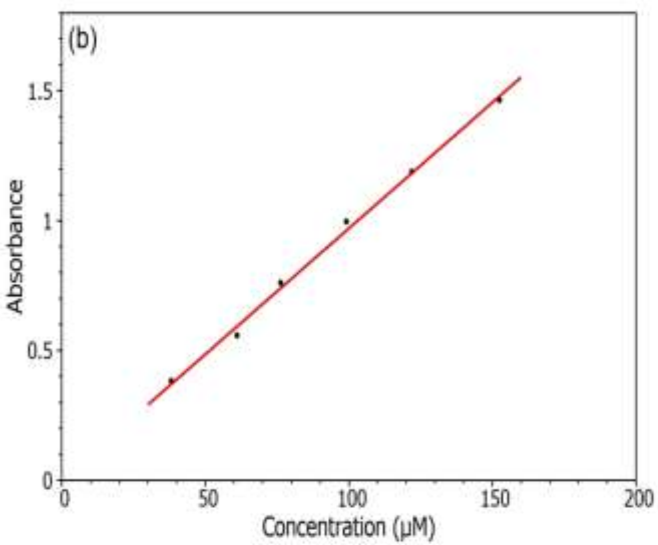
Beer-Lamberts law

Application

Absorption spectra: a linear calibration curve of the absorbance versus concentration







Beer‘s law follow if the following conditions are met:

- Incident radiation on the substance of interest is **monochromatic.**

- The solvent absorption is insignificant, compared with the solute absorbance.

- The solute concentration is within given limits.

- An optical interferant is not present.

- A chemical reaction does not occur between the molecule of interest and another solute or solvent molecule.

Deviations to the law

The Beer-Lambert law maintains linearity under specific conditions only.

The law will make inaccurate measurements at high concentrations
because the molecules of the analyte exhibit stronger intermolecular and electrostatics interactions which is due to the lesser amount of space between molecules.

- This can change the molar absorptivity of the analyte.

Not only does high concentrations change molar absorptivity, but it also changes the refractive index of the solution causing departures from the Beer-Lambert law.

What is a Spectrometer?

Any instrument that is used to measure the variation of a physical characteristic over a given range; i.e. a spectrum.

- **Mass Spectrometer :** Mass-to-charge ratio Spectrum
- **NMR Spectrometer :** Variation of nuclear resonant frequencies
- **Optical Spectrometer:** Change in the absorption and emission of light with wavelength

Spectrophotometers are classified as being either

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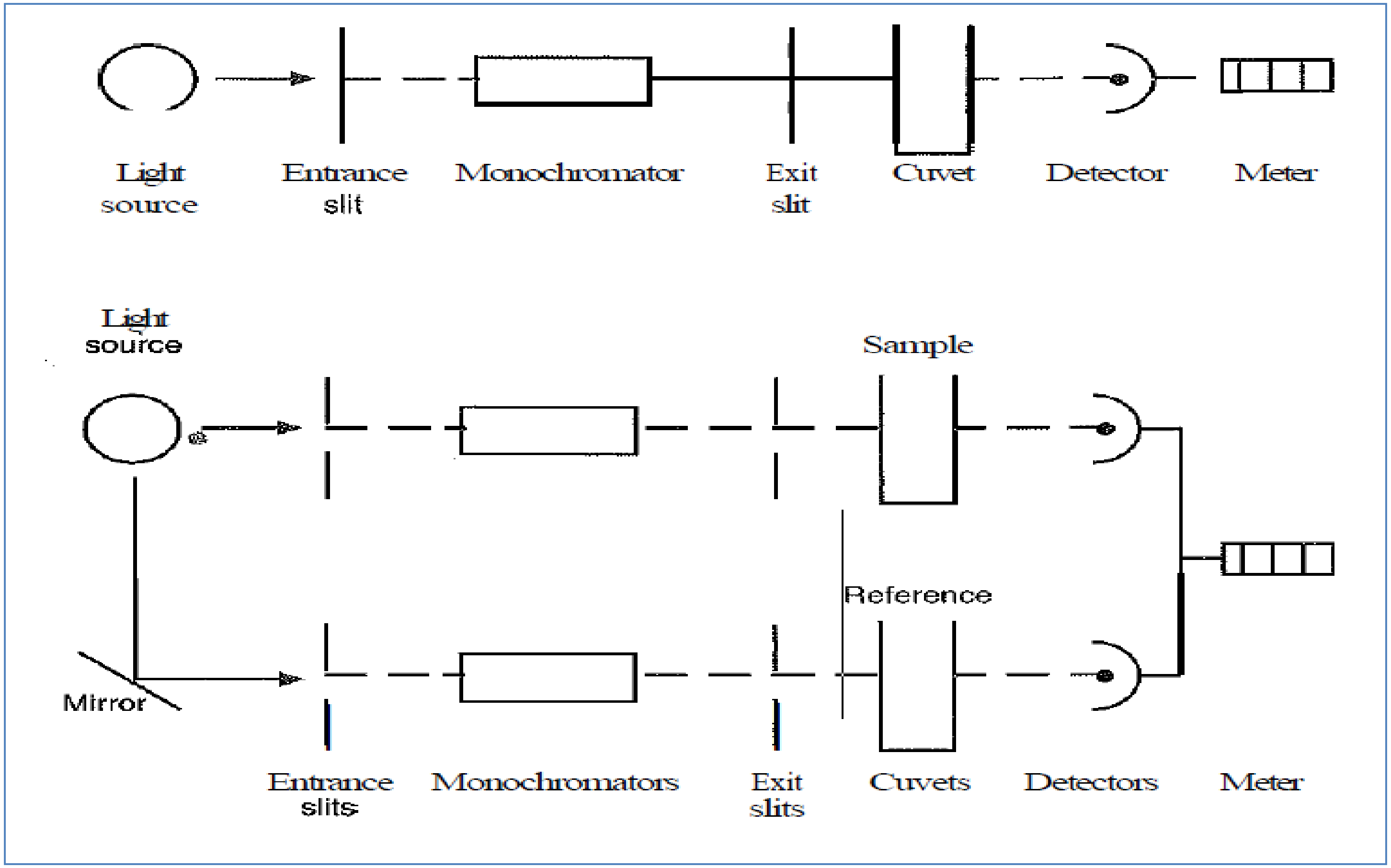
Single- Beam

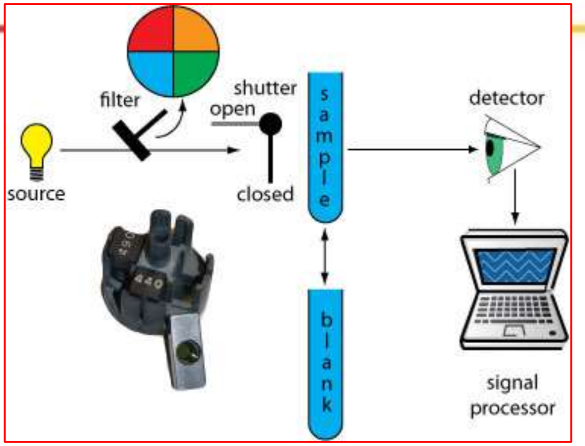
Double-Beam.



Optical Techniques

- ❑Photometry
- ❑Spectrophotometer
- ❑Flame Emission Spectrophotometery
- ❑Atomic Absorption Spectrophotometery
- ❑Fluorometry
- ❑Luminometry

Photometer and Spectrophotometer



- **Devices** used to measure intensity of light
  emitted by,
  passed through
  reflected by a substance

Photometry:

The measurement of the luminous intensity of light or the amount of luminous light falling on a surface from such a source.

- Defined originally as the process used to measure light intensity independent of wavelength.

- **Use Photometers filters**

Light Meter

Optical Spectrometer

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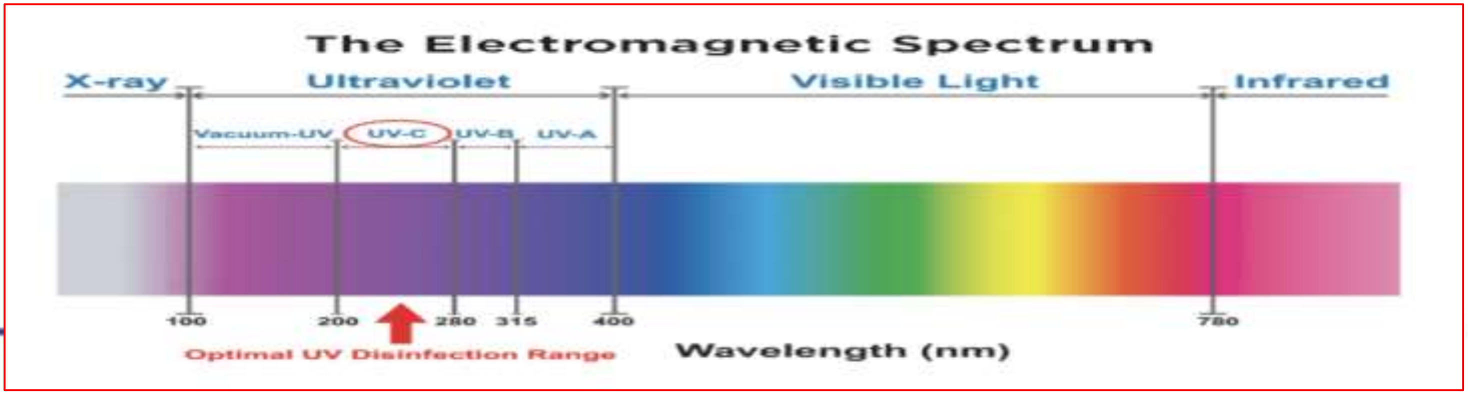
- **Goal:** Measure the interaction (absorption, reflection, scattering) of electromagnetic radiation with a sample or the emission (fluorescence, phosphorescence) of electromagnetic radiation from a sample.

- Optical spectrometers are concerned with electromagnetic radiation that falls within the **optical region of the electromagnetic spectrum. i.e** light spanning the ultraviolet (180-390nm), **Visible** (390-780nm) and **Infrared** (780- 12,000nm



Vibrant...

Spectrophotometry:

•The measurement of the intensity of light at selected wavelengths.

• Use prisms or Gratings

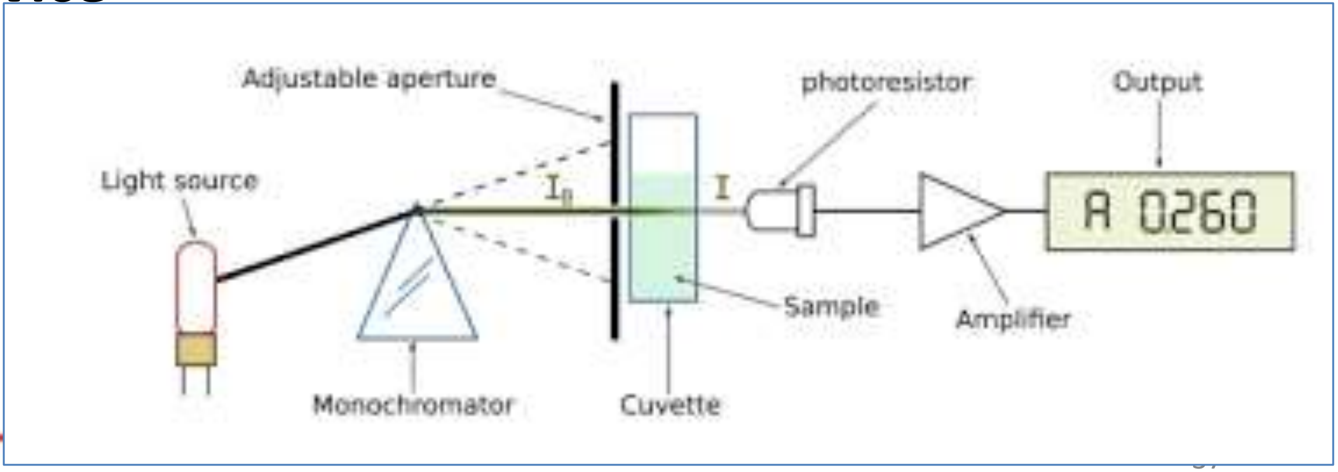
Ultraviolet (UV) radiation = <380 nm, The 180 to 390nm region of the electromagnetic spectrum.

Visible light radiation = The 390 to 780 nm region of the electromagnetic spectrum that is visible to the human eye.

Infrared (IR) Radiation: The 770 to 12,000nm region of the electromagnetic spectrum.

Spectrophotometer Components

1. **Light source:** Tungsten light bulb, Quartz-halogen lamp, laser Mercury-vapour lamp, hydrogen lamp, Deuterium lamp
2. **Slit**
3. **Monochromator:** Filters, Prisms and diffraction gratings, **Fiber optics**
4. **Cuvets:** Glass, Silica (quartz) and plastics
5. **A Photodetector:** Photomultiplier tube(PMT)
6. **A Readout device**
7. **A computer.**

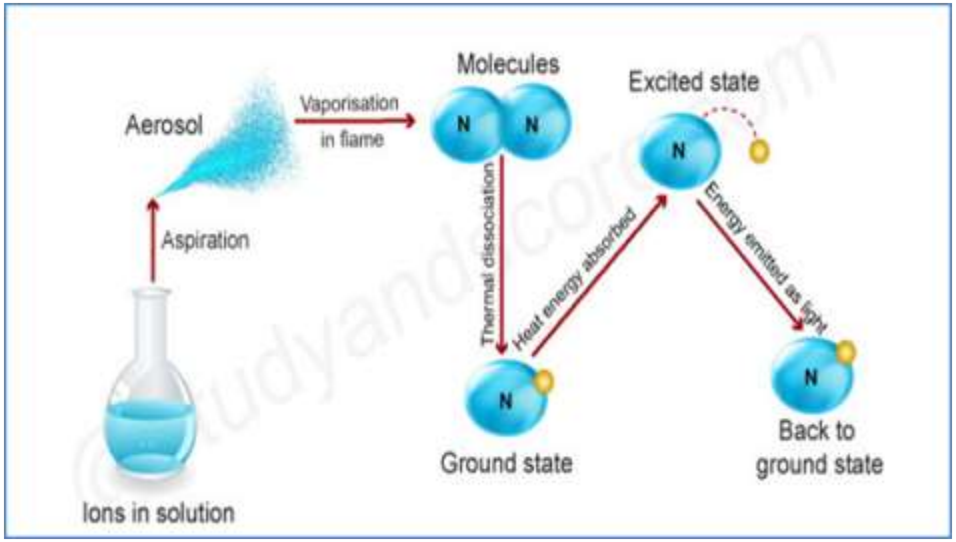


Flame Emission Spectrophotometry

- flame atomic emission spectrometry (FAES).
- A necessary tool in the field of analytical chemistry.
- Used to determine the concentration of certain metal ions like
  - Sodium,
  - Potassium,
  - lithium,
  - calcium,
  - cesium etc.

Principle of Flame photometer

- Compounds of the alkali and alkaline earth metals (Group II) dissociate into atoms when introduced into the flame.
- Some of these atoms further get excited to even higher levels. But these atoms are not stable at higher levels.



Principle of Flame photometer

- These atoms emit radiations when returning back to the ground state. These radiations generally lie in the visible region of the spectrum.
- Each of the alkali and alkaline earth metals has a specific wavelength.

Element	Emitted wavelength	Flame color
Sodium	589 nm	Yellow
Potassium	766 nm	Violet
Barium	554 nm	Lime green
Calcium	622 nm	Orange
Lithium	670 nm	Red

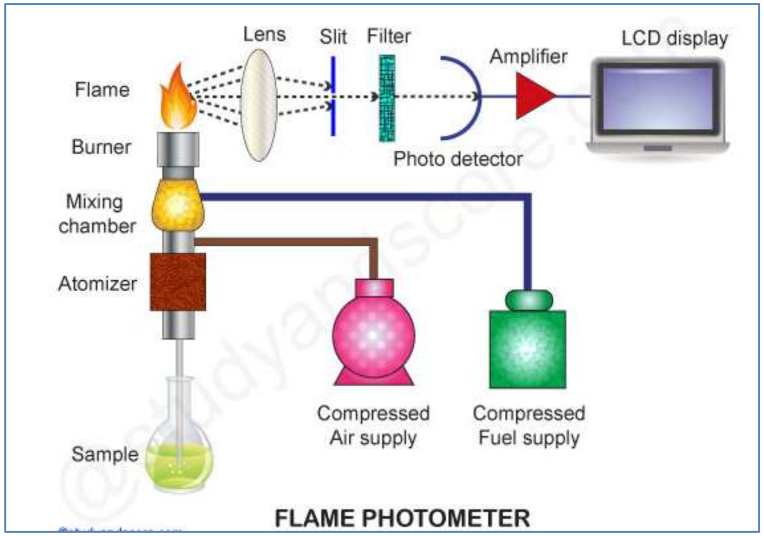
Principle of Flame photometer

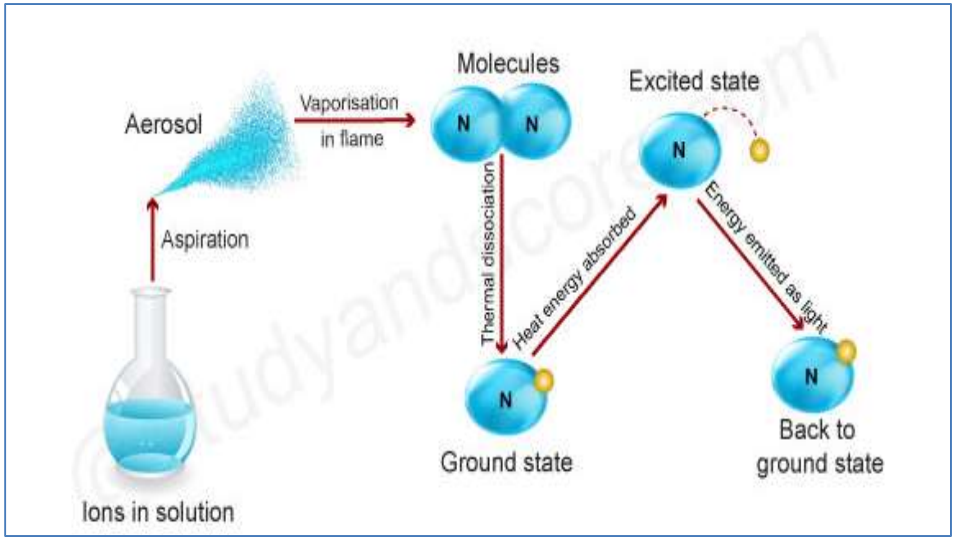
- These colors are characteristic of the metal atoms that are present as cations in solution.

- The light intensity of the characteristic wavelength produced by each of the atoms is directly proportional to the number of atoms that are emitting energy, which in turn is directly proportional to the concentration of the substance of interest in the sample.

Principle of Flame photometer

- It is based on the measurement of the emitted light intensity when a metal is introduced into the flame.
- The wavelength of the colour gives information about the element and the colour of the flame gives information about the amount of the element present in the sample





https://www.studyandscore.com/studymaterial-detail/flame-photometer-principle-components-working-procedure-applications-advantages-and-disadvantages

Branch of Atomic Absorption Spectroscopy. **Flame photometer**

Desolvation:

Desolvation involves drying a sample in a solution. The metal particles in the solvent are dehydrated by the flame and thus solvent is evaporated.

Vaporization: The metal particles in the sample are also dehydrated. This also led to the evaporation of the solvent.

Atomization: Atomization is the separation of all atoms in a chemical substance. The metal ions in the sample are reduced to metal atoms by the flame.

Excitation: The electrostatic force of attraction between the electrons and nucleus of the atom helps them to absorb a particular amount of energy. The atoms then jump to the higher energy state when excited.

Emission: Since the higher energy state is unstable the atoms jump back to the ground state or low energy state to gain stability. This jumping of atoms emits radiation with characteristic wavelength. The radiation is measured by the photo detector.

❑ Limitation of the Flame Photometer

- The element is not appreciably excited in the flame, but is merely dissociated from its chemical bonds (atomized) and placed in an unexcited or ground state (Neutral atom)

- This unexcited atom is capable to absorbing radiation at a specific wavelength.

- For example: if the cathode lamp of Na was made, sodium light (589nm) will be emitted by the lamp.
- When light enters in the flame, some of it is absorbed by ground state atoms in the flame.
- Results in a net decrease in the intensity of the lamp beam

Atomic Absorption Spectrophotometry (AAS)

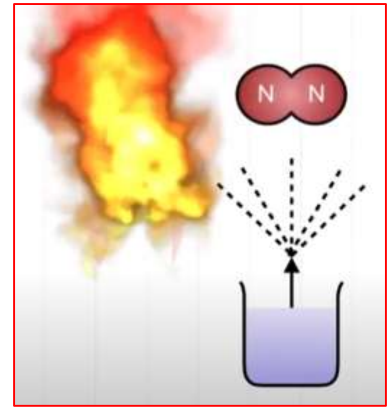
- Analysis technique for rapid trace metal analysis
- It is based on element specific wavelength light absorption by ground state atoms in the flame or electro thermal graphite furnace.

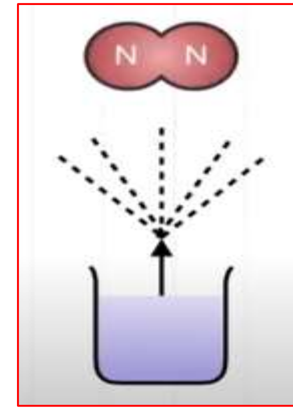
Atomic Absorption Spectrophotometry (AAS)

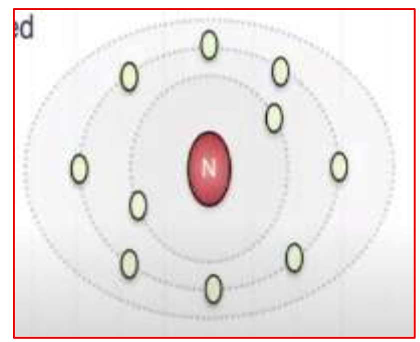
- When the salt soln. is put into the flame first solvent is vaporized the tiny particles of solute molecules are obtained which on further heating in the flame are converted into gaseous molecules the molecules dissociate into atoms.

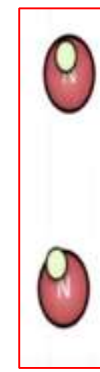
- Some of the atoms are exited unexcited atoms will absorb their characteristic radiation.

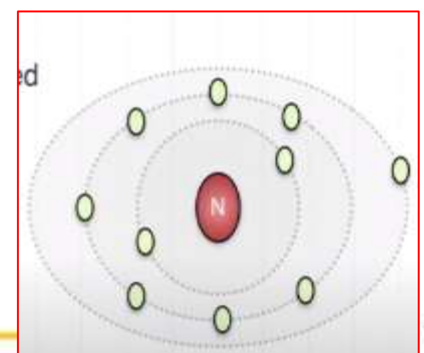
- A.A.S. is concerned with this part of light (radiations) which are absorbed by the unexcited atoms present in the ground state.



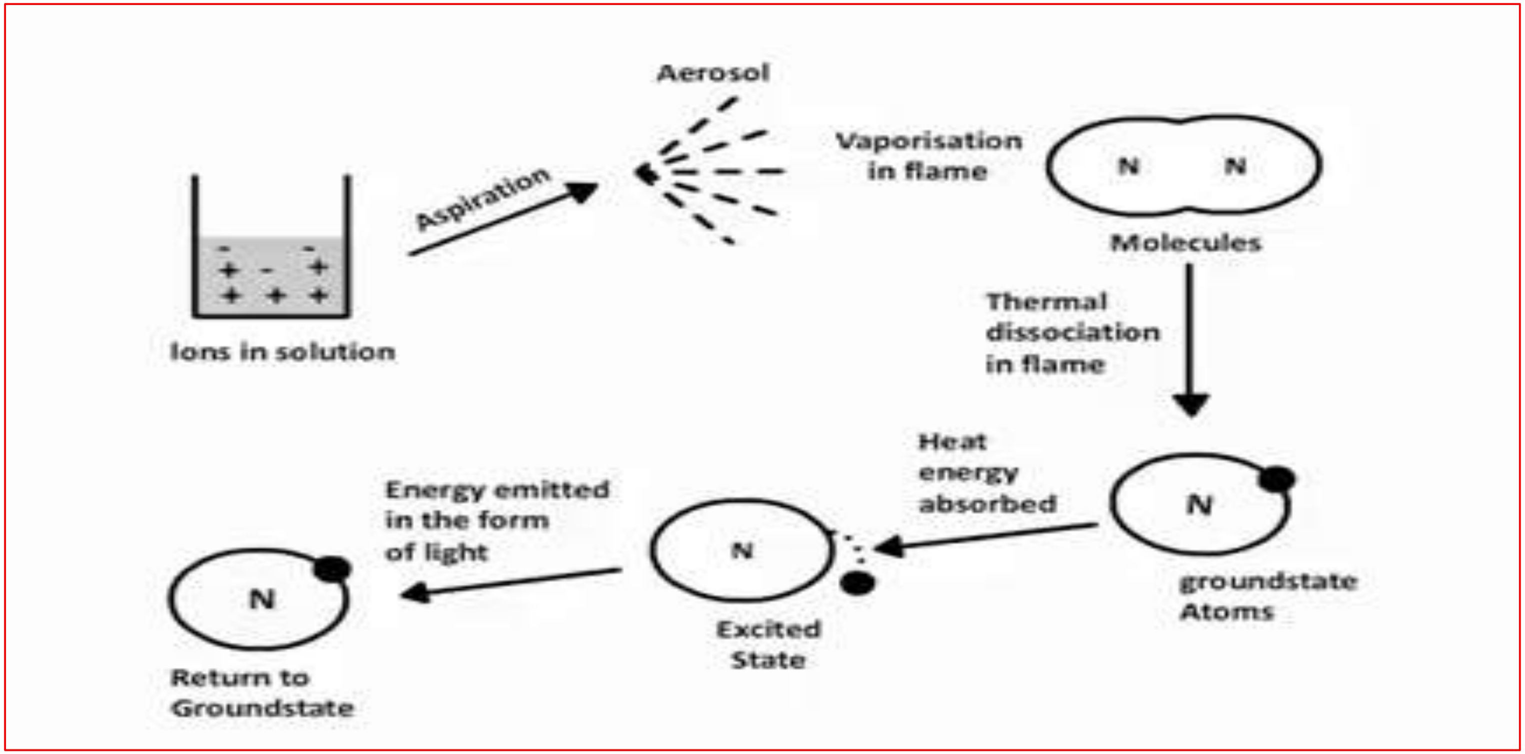








Atomic Absorption Spectrophotometry (AAS)



Applications

- Determination of even small amounts of metals (lead, mercury, calcium, magnesium, etc.)

- Environmental studies:
  Drinking water, ocean water, soil

- Food industry.

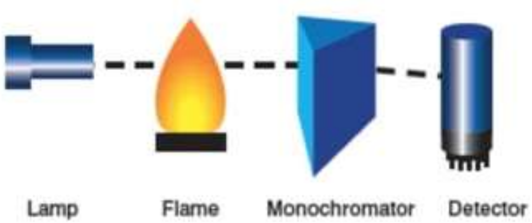
- Pharmaceutical industry.

ATOMIC SPECTROSCOPY

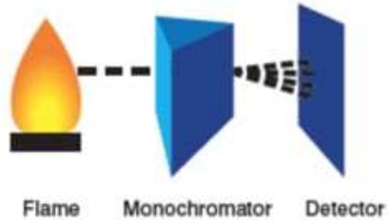
Absorption Spectroscopy
AAS

Emission Spectroscopy
FES, ICP-AES(OES)

ATOMIC ABSORPTION



ATOMIC EMISSION



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Thank You!