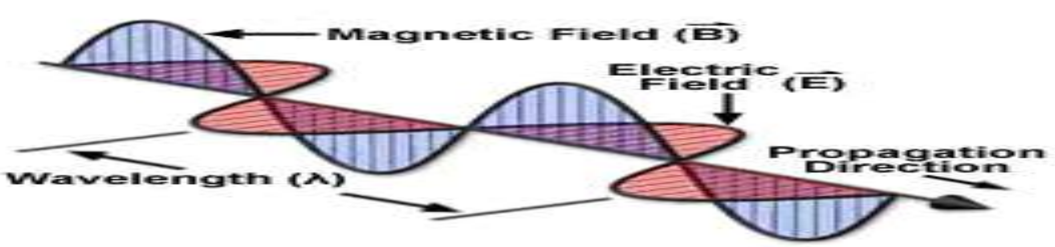
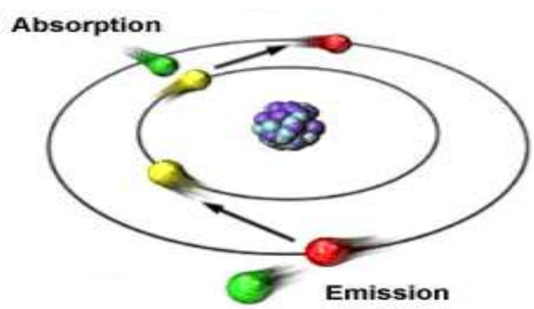
**Department of Biochemistry/ Second Year – Laboratory Technique**

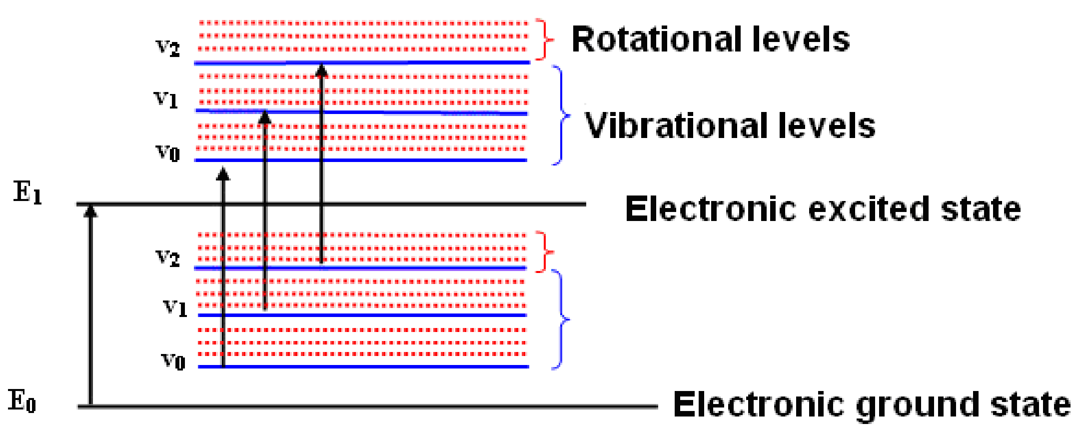
**Lecture Title: Atomic Absorption, Spectroscopy, spectrometry? / Lecture: Abbas**

# What is AAS/Atomic Absorption Spectroscopy?

**Atomic absorption spectroscopy(AAS) and atomic emission spectroscopy(AES)** is a electroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation by free atoms in the gaseous state.

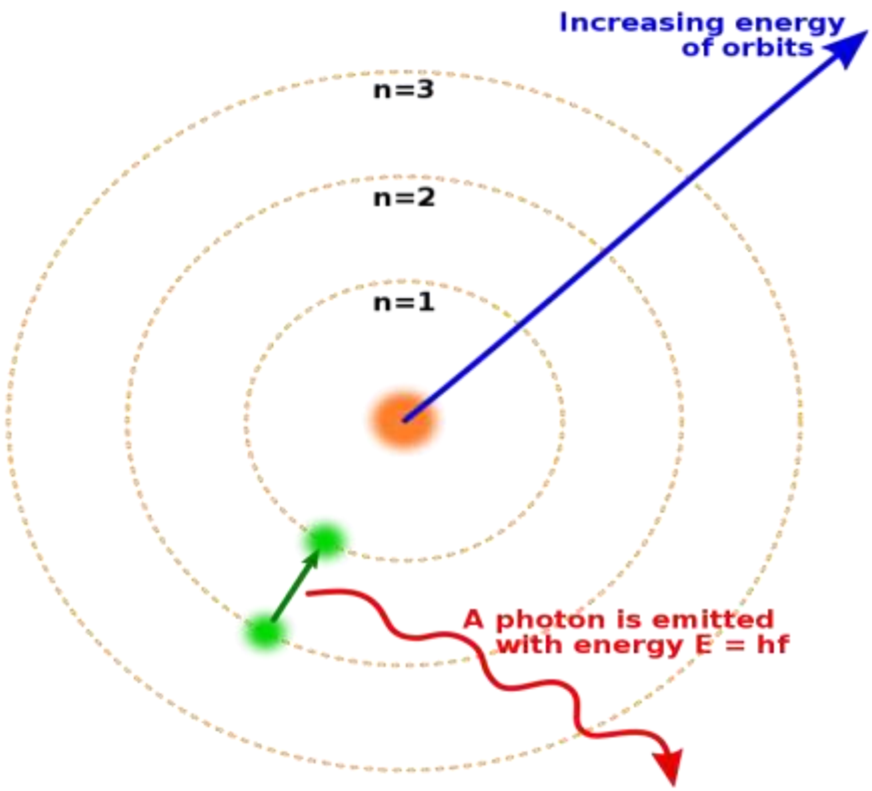


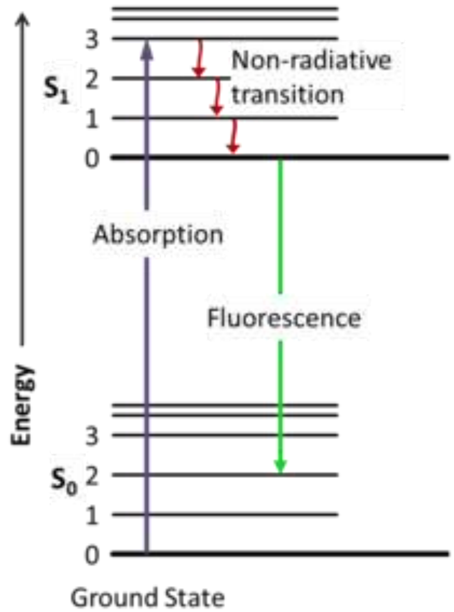
What is atomic absorbance AAS and atomic emission AES?
**Atomic absorption spectrometry** (**AAS**) is an analytical technique that measures the concentrations of elements. Atomic **absorption** is so sensitive that it can measure down to parts per billion of a gram (µg dm–3) in a sample
The technique makes use of the wavelengths of light specifically absorbed by an element.

**Atomic Emission Spe**




**(AES)**.: Atomic emission occurs when a valence electron in a higher energy atomic orbital returns to a lower energy.

**Atomic absorption spectroscopy** (**AAS**) is based upon the **principle** that free **atoms** in the ground state can absorb light of a certain wavelength. **Absorption** for each element is specific, no other elements absorb this wavelength.

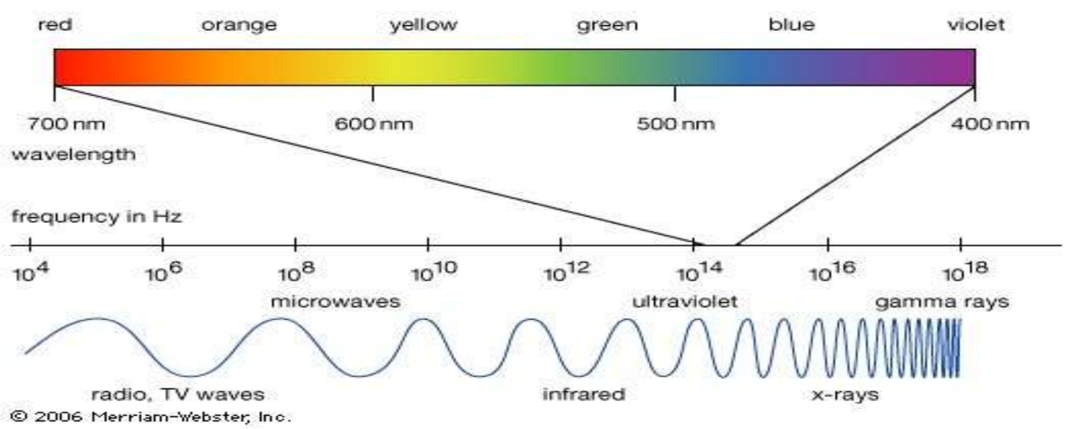



**Atomic absorption spectroscopy**, or **AAS**, is a technique for measuring the concentrations of metallic elements in different materials. ... Distinct elements will absorb these wavelengths differently

What is the difference between AAS and AES?
While **AAS** quantifies the absorption of electromagnetic radiation by well-separated neutral atoms, **AES** measures emission of radiation from atoms in excited states.

What is the principle of absorption?

Atomic absorption methods measure the amount of energy (in the form of photons of light, and thus a change in the wavelength) absorbed by the sample. Specifically, a detector measures the wavelengths of light transmitted by the sample (the "after" wavelengths), and compares them to the wavelengths, which originally passed through the sample (the "before" wavelengths). A signal processor then integrates the changes in wavelength, which appear in the readout as peaks of energy absorption at discrete wavelengths




What are the application of AAS?
**AAS** can be used to determine over 70 different elements in solution, or directly in solid samples via electro thermal vaporization, and is used in pharmacology, biophysics, archaeology and toxicology research.

Is absorption a chemical reaction?
**Chemical absorption** or reactive **absorption** involves a **chemical reaction** between the **substance** being **absorbed** and the **absorbing** medium. In some cases, it occurs in combination with physical **absorption**. **Chemical absorption** depends upon the stoichiometry of the **reaction** and the concentration of the reactants

What are the mechanisms of absorption?
Absorption can occur through five mechanisms: (1) active transport, (**2**) passive diffusion, (3) facilitated diffusion, (4) co-transport (or secondary active transport), and (5) cytolysis.

## What is flame atomic absorption spectroscopy?

**Flame Atomic Absorption Spectrometry** is a sensitive technique for the quantitative determination of more than sixty metals. As it is used for determining the concentration of metals it can be applied in Environmental Analysis. It can also be used to detect if there is trace metals present in food.

What is absorption frequency?

An **absorption** band is a range of wavelengths, **frequencies** or energies in the electromagnetic spectrum which are characteristic of a particular transition from initial to final state in a substance.

How is atomic absorption spectroscopy used?

**Atomic absorption** spectrometers **use** the **absorption** of light to measure the concentration of gas phase **atoms**. The light that is focused into the flame is produced by a hollow cathode lamp, inside which is the sample and an anode

Why is AAS useful for metal analysis?

In **atomic absorption spectroscopy** (**AAS**) element specific light absorption is **used** to determine the concentration of a **metal** in a solution. This mixture is composite to reduce the elements of interest into free atoms that absorp light at a specific wavelength.

Where is AAS used?

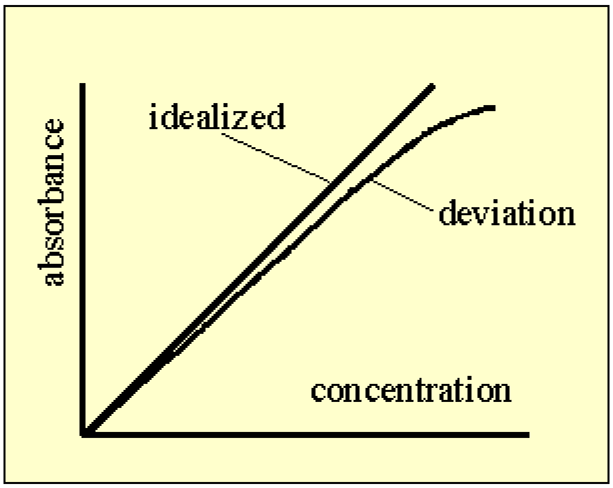
**Atomic absorption spectrometry** (**AAS**) is an easy, high-throughput, and inexpensive technology **used** primarily to analyze compounds in solution. As such, **AAS** is **used** in food and beverage, water, clinical, and pharmaceutical analysis.

What is the process of AAS?

The process of atomic absorption spectroscopy (AAS) involves two steps:

1. Atomization of the sample
2. The absorption of radiation from a light source by the free atoms

The sample, either a liquid or a solid, is atomized in either a flame or a graphite furnace. Upon the absorption of ultraviolet or visible light, the free atoms undergo electronic transitions from the ground state to excited electronic states.




What is spectroscopy and why is it important?
**Spectroscopy** is used as a tool for studying the structures of atoms and molecules. The large number of wavelengths emitted by these systems makes it possible to investigate their structures in detail, including the electron configurations of ground and various excited states.

What is difference between spectroscopy and spectrophotometry?
You can think of **Spectrometry** as general study of interaction of matter with electromagnetic waves (the whole spectra). While **Spectrophotometry** is the quantitative measurement of light spectra reflection and transmission properties of materials as function of the wavelength.

Types of atomic absorption :

1-Flame AAS (Nebulization champers) Fuel and oxide gases .

2- Flameless AAS :

A-Electrothermal AAS.

B-Cold Atomization AAS.

Instrumentation ----What is the component of AAS?

1-Atomizers(Nebulizer):-

A-Flame atomizers the air-acetylene flame with a temperature of about 2300 °C and the nitrous oxide system ($N_2O$)-acetylene flame with a temperature of about 2700 °C.

B- Electro thermal atomizers (*using graphite tube atomizers*)Flameless.

2-Radiation sources

A-Hollow cathode lamps(HCL)(190-800)nm. B-Deuterium lamps. C-Xenon lamps.

3-monochromator.(prism, Interferon Filter, Diffraction grating )

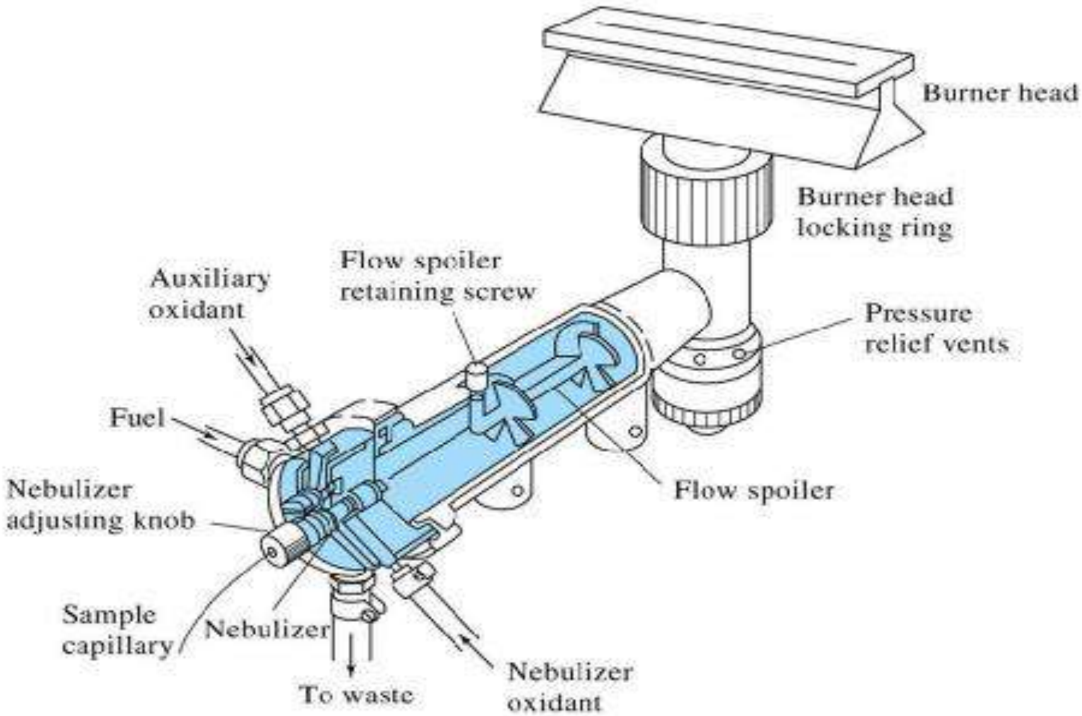
4-detector. 5-Data processer

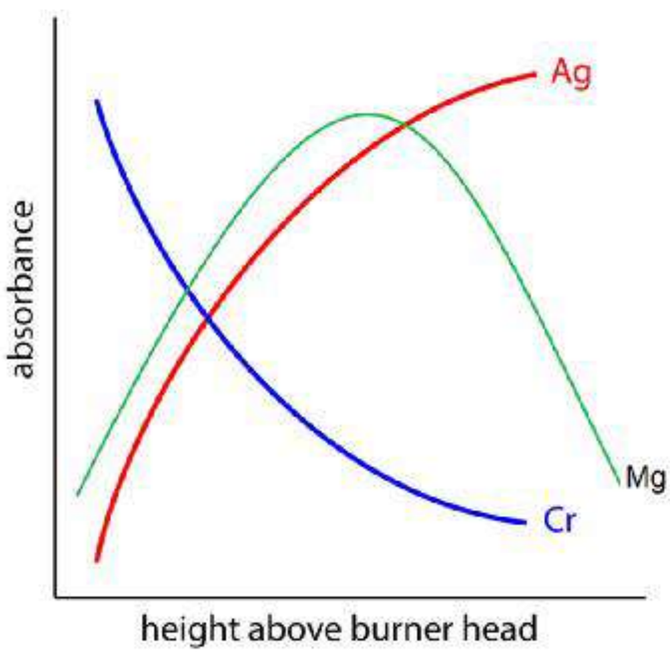
Steps of Atomization :

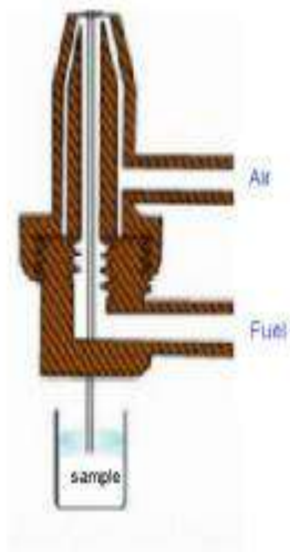
1-Dry step (100 C) .

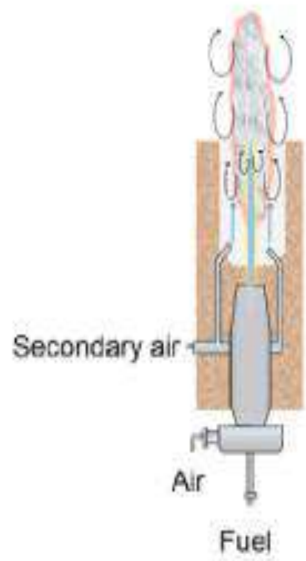
2-Ash step (150- 1500 )C .

3- Atomize step (1100 – 3000)C.


Burner head
Burner head locking ring
Flow spoiler retaining screw
Auxiliary oxidant
Pressure relief vents
Fuel
Flow spoiler
Nebulizer adjusting knob
Sample capillary
Nebulizer
To waste
Nebulizer oxidant

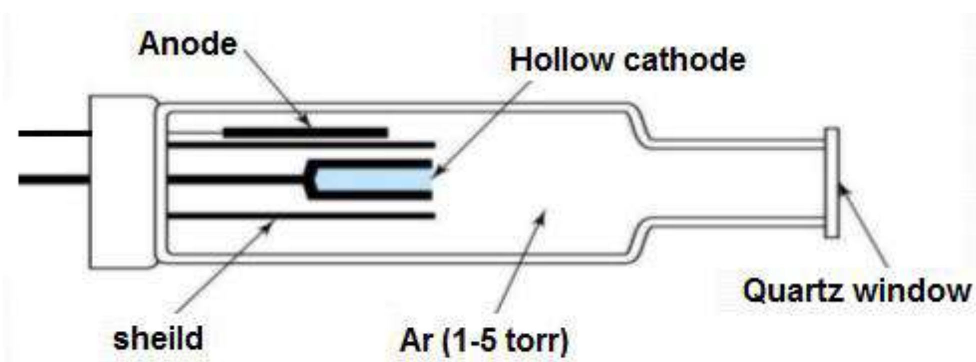

Ag
Mg
Cr
absorbance
height above burner head



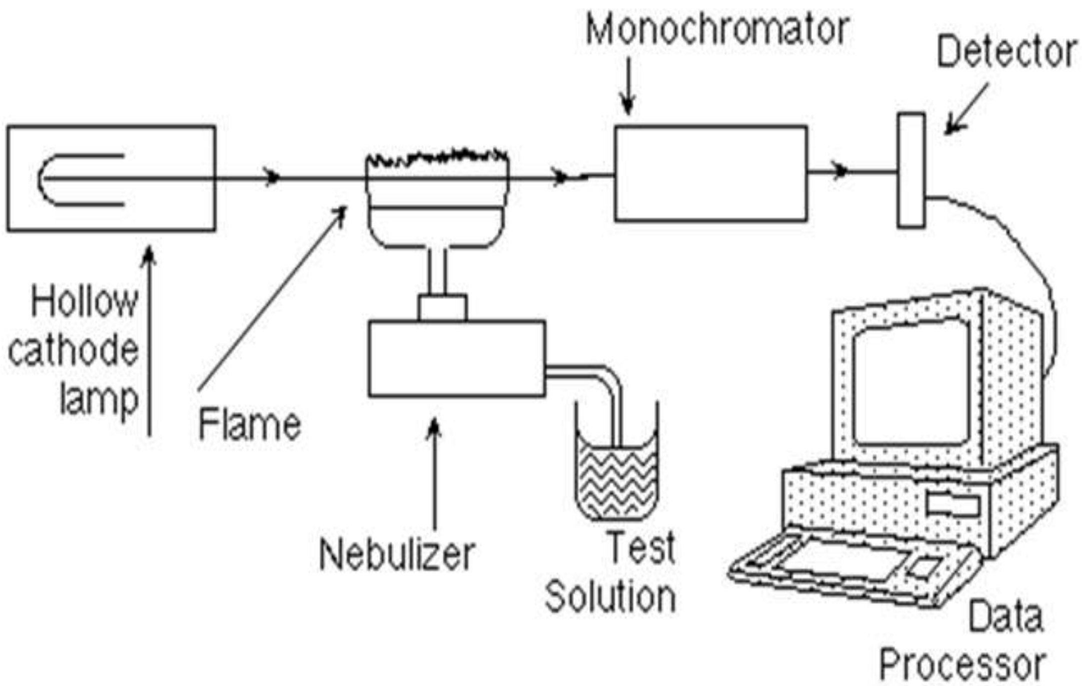




**Sources of radiation:-**

1- **Hallow cathode lamp (HCL)containing Ar and Ne gas in (1-5)torr, also anode and cathode from the same element in (4-100)Am.**
2- **Electrode discharge lamp (diselectrode).**



What is the component of AAS?




شكل يوضح طريقة التحليل بالامتصاص الذرى .

How do you use Beer's Law equation?
The **Beer**-Lambert **law** relates the absorption of light by a solution to the properties of the solution according to the following **equation**: A = εbc, where ε is the molar absorptivity of the absorbing species, b is the path length, and c is the concentration of the absorbing species.

**Calculation** of **concentration** (C = A/(L x Ɛ))

What is the difference between Lambert law and beer law?
**Lambert's law** stated that the loss of light intensity when it propagates **in a** medium is directly proportional to intensity and path length. ... **Beer's law** stated that the transmittance of a solution remains constant if the product of concentration and path length stays constant.

What is the formula of parts per thousand, million and billion?

ppt= (gram of solute) / (liters of **solution**) × $10^3$

ppm = (gram of solute) / (liters of **solution**) × $10^6$

ppb = (gram of solute) / (liters of **solution**) × $10^9$

1. One part per million is equivalent to 1 minute in:- a. 1 day b. 2 years c. 6 weeks

2. One part per billion is equivalent to 1 second in:- a. 3 weeks b. 17 months c. 32 years

365 days/ 1 year x 24 hours/ 1 day = 8,760 hours/ 1 year

8,760 hours /1 year x 60 minutes /1 hour = 525,600 minutes/ 1 year

525,600 minutes/ 1 year x 60 seconds /1 minute = 31,536,000 seconds/ 1 year

## Principle of Atomic Absorption Spectroscopy

Atomic absorption spectroscopy (AAS) is based upon the principle that free atoms in the ground state can absorb light of a certain wavelength. These very specific wavelengths give the technique excellent specificity and detection limits in the AAS analysis. Absorption for each element is specific, no other elements absorb this wavelength. Typical applications of AAS include –

- Quantitative metal concentrations in solution
- Analysis of lead in paint
- Monitoring of trace metals in industrial effluent streams
- Analysis of additives and purity in steels and other metal alloys
- Analysis of low-level contaminates.

**Wave properties:-**

**1- Wavelength ($\lambda$)**

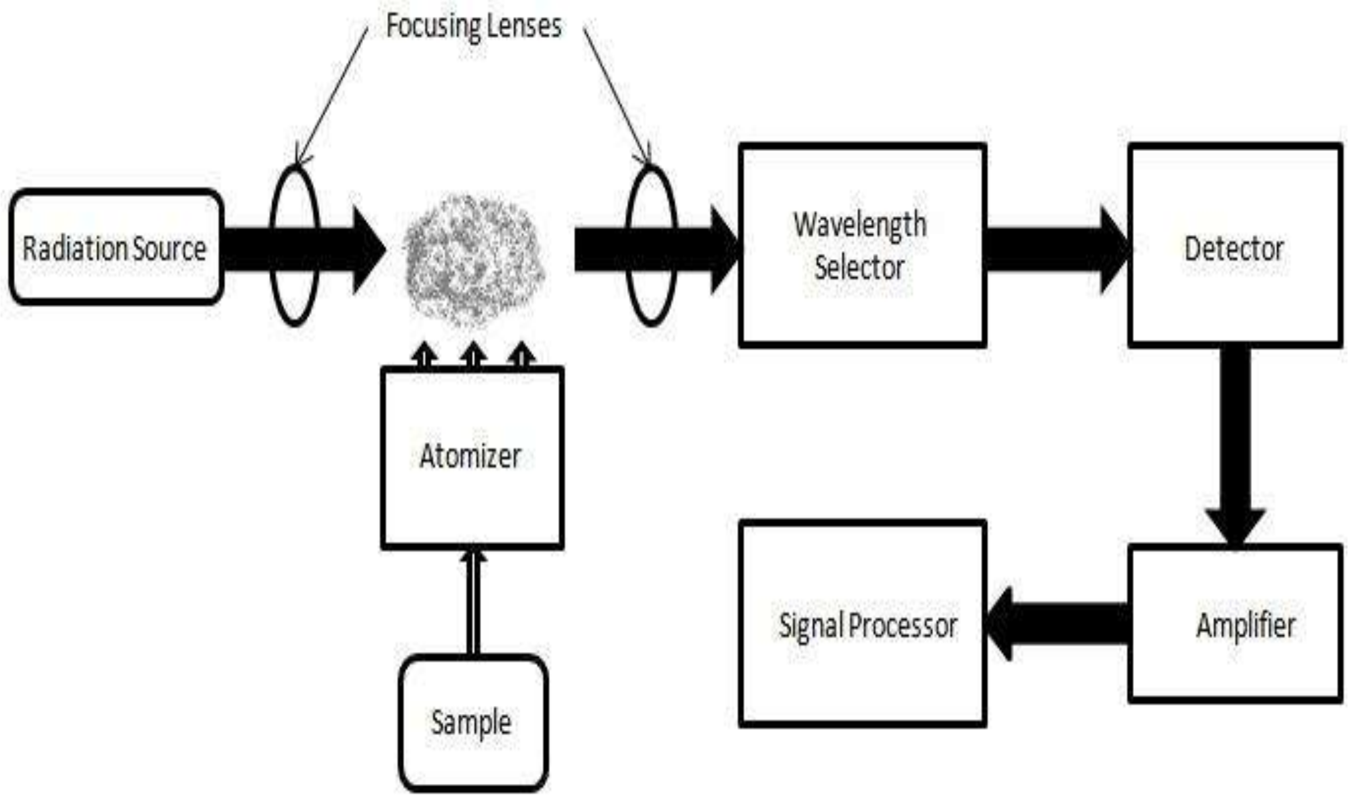
**2-Frequency ($\nu$) ,** Hz

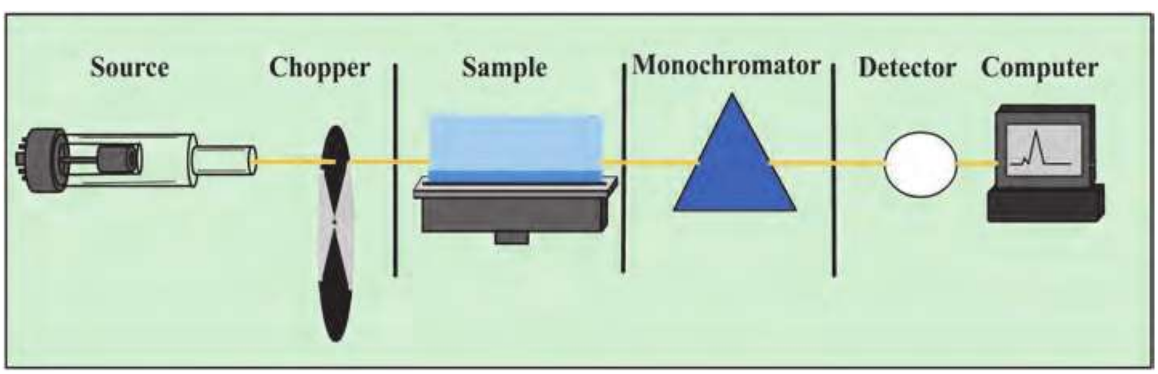
**3-Wave number ($\bar{\nu}$)** = 1 / $\lambda$ (cm) = $cm^{-1}$

$E = h\nu$ , $c = \lambda \nu$ , $\nu = c/\lambda$ , $E = hc/\lambda$

$h = 6.624 \times 10^{-27}$ erg / sec

$c = 2.99 \times 10^{10}$ cm/sec




**Interferences in atomic absorption:-**

**1- Matrix interferences (viscosity, surface tension) effect in number of free atoms .**

**2-Chemical interferences (type of bonds)[Ca3(po4)]that have very strong bond.**

**3-Ionization interferences (High temperature)to treat this case addition ionization buffer for example (NaCl , CsBr)mater ionized very easy .**

**4-Spectral interferences (same spectral) for example Cu=324.754nm , Er=324.753nm.**

**Q/Calculate the amount of energy for an electron when transfer from 5 shall to 3 shall and calculate the wave length for this emission radiation ?**

**Solution**

$$E= -R_H\,(1/(n_f)^2\ -\ 1/(n_i)^2\,)$$

$$E= -2.18 \times 10^{-18}\ J\ (1/\ (3)^2\ -\ 1/(5)^2\ )$$

$$E= 1.55 \times 10^{-19}\ J$$

$$E = h\ c\ /\lambda \quad ------ \quad \lambda = h\ c\ /E$$

$$\lambda = 6.63 \times 10^{-34}\ J.S \times 3 \times 10^{8}\ m/s \quad / \quad 1.55 \times 10^{-19}\ J$$

$$\lambda = 1.28 \times 10^{-6}\ m$$

$$\lambda = 1280\ nm$$

1 nm = $10^{-9}$ m = $10^{-7}$ cm.

1 µm = $10^{-6}$ m = $10^{-4}$ cm

1A° = $10^{-10}$ m = $10^{-8}$ cm

1nm = 10 A°

1 µm = 10000 A°

**Transmitted:-**

$T = I_i / I_\circ$ -----  $A = \log I_\circ / I_i$

$I_i$= transmitted beam (Permeative beam)

$I_\circ$=Incident beam

$1/T = I_\circ / I_i$ ----- $\log 1/T = \log I_\circ / I_i$ --- $\log 1/T = A$

$A = -\log T$

$\%T = I_i / I_\circ \times 100$

Q1/Calculate the Absorption for solution have (T=30%) in $\lambda$=640nm?

Solution -----

T=30%--------T=30/100-----T=0.3

A=-logT ----A=-log0.3 =0.5228

Q2/Absorption cell that have length (1cm) containing (0.05 mol/L) concentration of solution , when give beam in $\lambda$=280nm the absorption for this solution is (A=1.5).

Calculate molar absorptivity ( $\varepsilon$ ) for this solution?

Solution -----

$A = \varepsilon bc$ ----- $\varepsilon = A/bc$

$\varepsilon = 1.5/1\times0.05 = 30\ L\ .\ mol^{-1}\ .cm^{-1}$

**Good luck**