RAMA UNIVERSITY

www.ramauniversity.ac.in

DEPARTMENT OF BIOTECHNOLOGY
FACULTY OF ENGINEERING & TECHNOLOGY

LT 24. Atomic Absorption & Emission spectroscopy

Content Outline

1. AAS : Principle
2. Sensitivity
3. AES: principle
4. Applications
5. Reference & Further reading

Atomic Absorption Spectroscopy : Principle

- Atomic Absorption (AA) is based on the principle that a **ground state atom** is capable of absorbing light of the same **characteristic wavelength** as it would emit if excited to a higher energy level.
- In flame AA, a cloud of ground state atoms is formed by aspirating a solution of the sample into a flame of a temperature sufficient to convert the element to its **atomic state**.
- The degree of absorption of characteristic radiation produced by a suitable source will be proportional to the population of ground state atoms in the flame, and hence to the **concentration of the element** in the **analyte**.

Compound $\xrightarrow{\text{Heat}}$ Atoms

Spectra of atoms consist of **SHARP LINES**.

Each element has a characteristic spectrum.

Due to sharpness of lines, there is little overlap between the spectral lines of different elements.

Therefore, there is little interference.

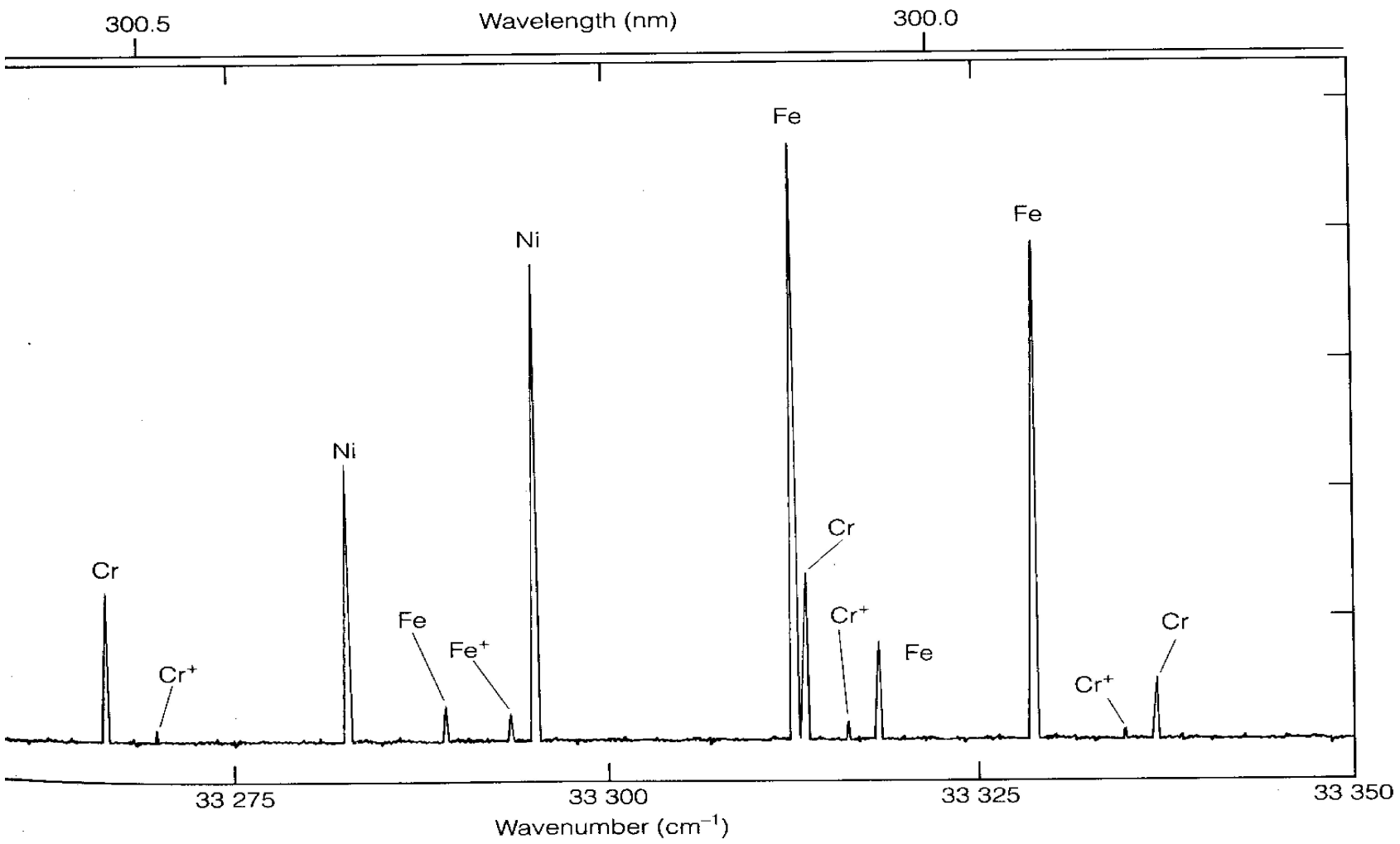
Atomic Spectroscopy

Sample $\xrightarrow[\text{Temperature}]{\text{High}}$ Vapour

Measure absorbance or emission of the atomic vapour.

Atomic spectroscopy deals with atoms.

Fe^{2+} and Fe^{3+} will not be distinguished.


300.5
Wavelength (nm)
300.0
Fe
Ni
Fe
Ni
Cr
Cr
Fe
Cr+
Fe+
Cr+
Fe
Cr+
Cr
33 275
33 300
33 325
33 350
Wavenumber (cm−1)

Sensitivity

- Atomic spectroscopy is very sensitive for most elements.
- Concentrations at the ppm level may be routinely determined using flame atomisation.
- Using electrothermal atomisation, concentrations at the ppb may be determined.
- 1 ppm = 10^{-6}g/g or 1μg/g
- The density of dilute aqueous solutions is approximately 1.00 so that:

1 μg/g of aqueous solution = 1μg/ml = 1 ppm

1 ppm Fe = 1 x 10^{-6} g Fe/ml = 1.79 x 10^{-5} mol dm^{-3}

1 ppm = 1 second in 11.6 days

1 ppb = 1 second in 31.7 years.

Atomic Absorption Spectroscopy

Absorbance = $-\log(I_t/I_0)$

I_o = incident radiation (on sample)

I_t = transmitted radiation.

Atomic Emission Spectroscopy

Absorbance = $-\log(I_t/I_0)$

I_0 =intensity of radiation reaching detector in the absence of sample.

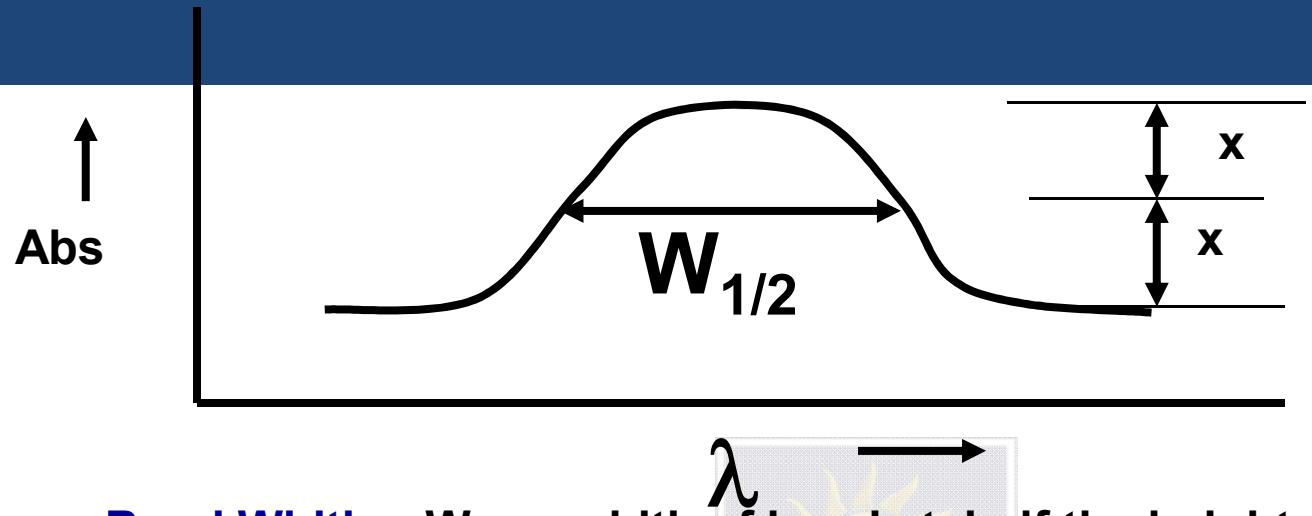
I_t = intensity of radiation reaching detector when sample is being aspirated.

- Both methods are used to determine the concentration of an element in solution.
- Both methods use a **standard curve**.
- Difference between **UV** and **IR** spectroscopy is that sample must be **atomised**.
- Sample may be atomised by:

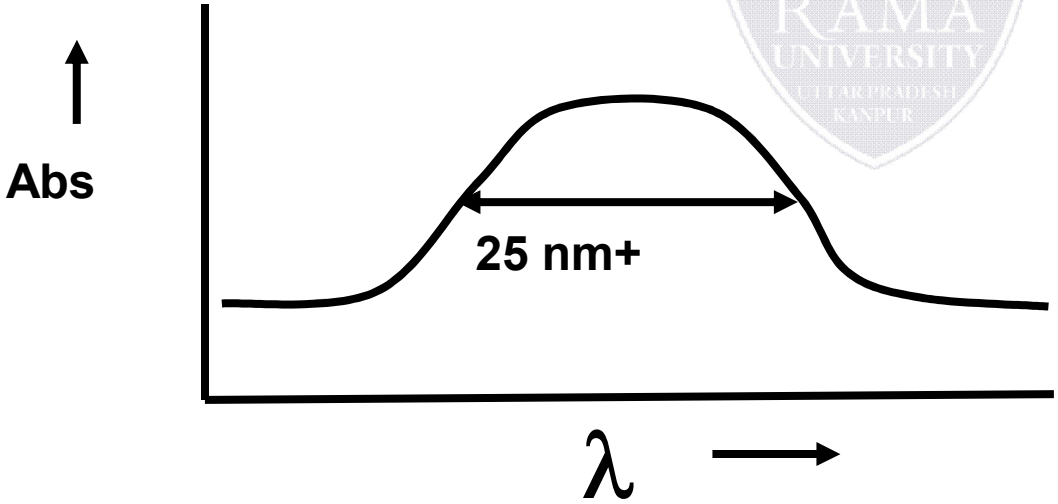
  (1) A flame

  (2) Electrically heated furnace

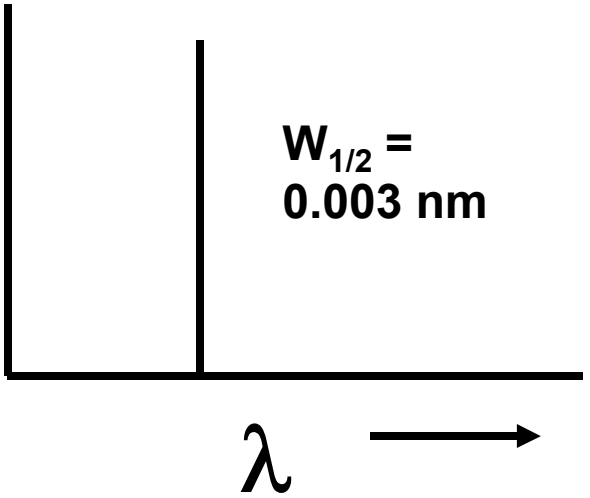
  (3) A Plasma



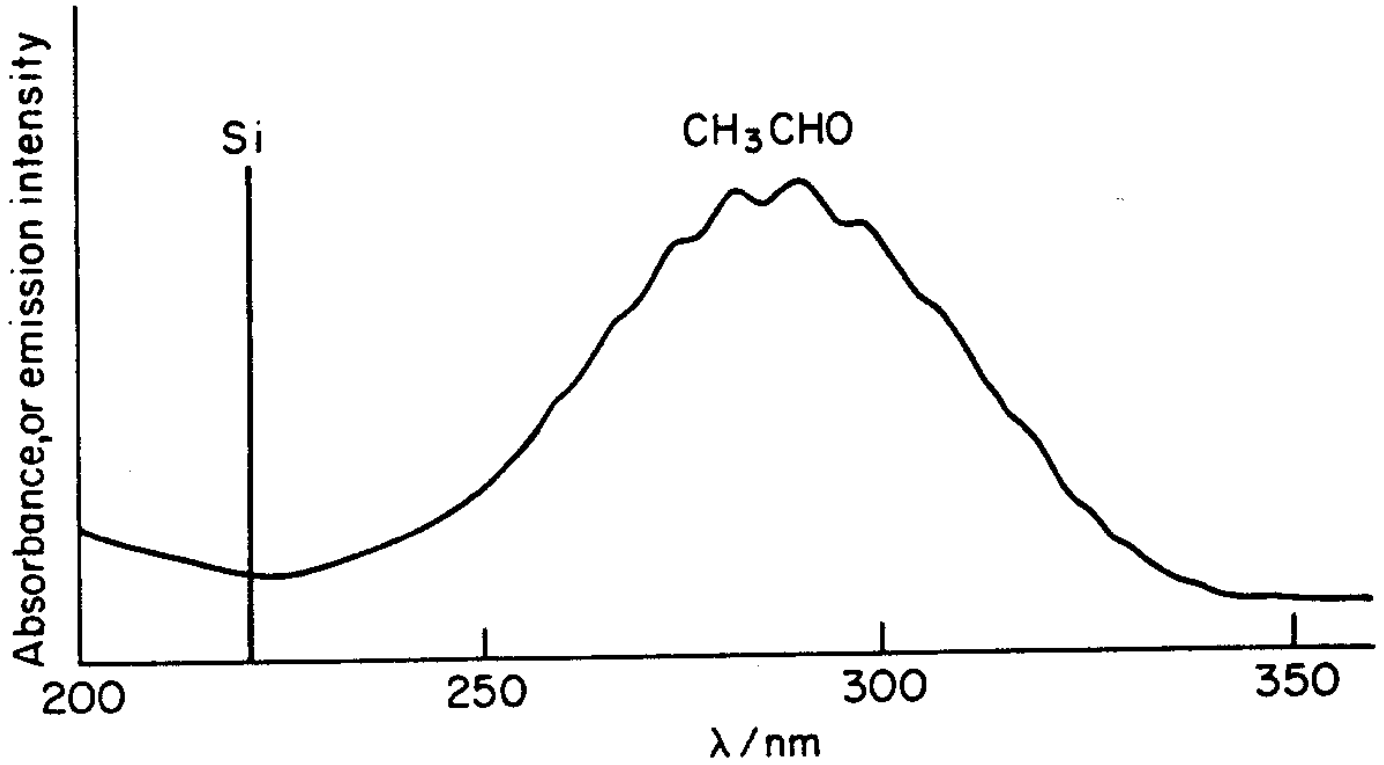
Band Width = $W_{1/2}$ = width of band at half the height



Molecular Absorption Band



Atomic Vapour Absorption Band



Comparison of the atomic emission spectrum of silicon compared with the molecular absorption spectrum of ethanal.

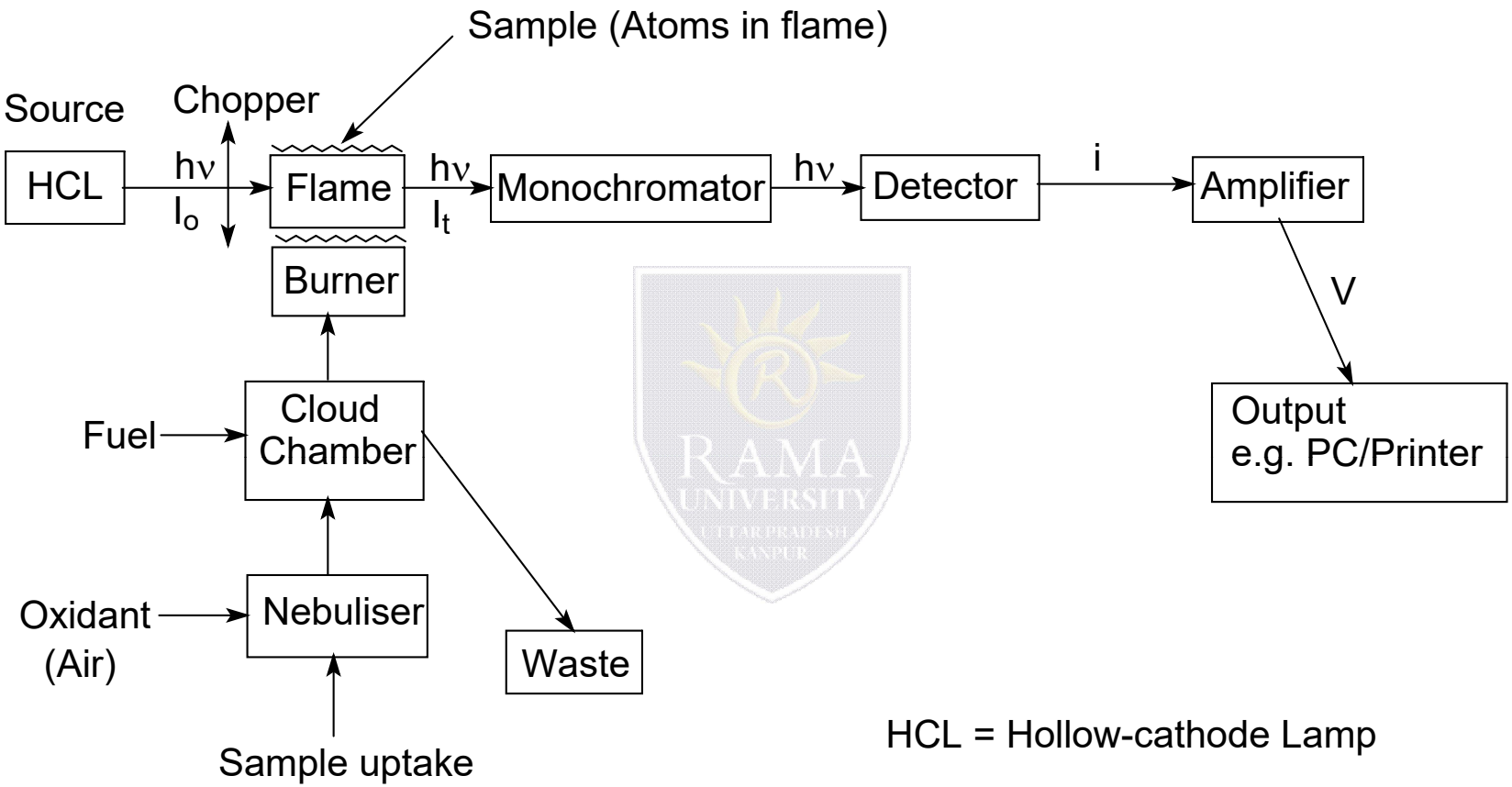
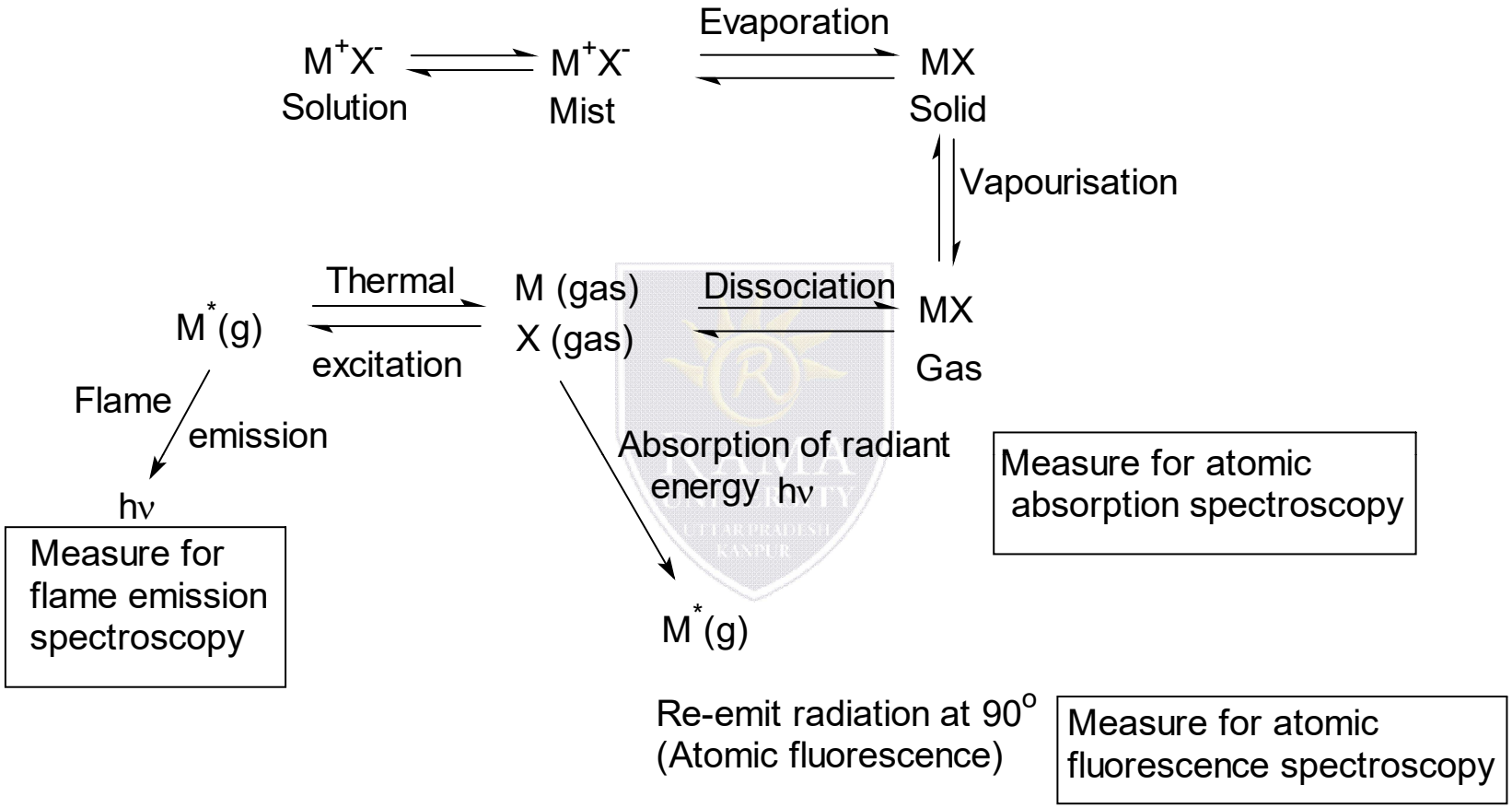


Figure 1. Schematic Diagram of an Atomic Absorption Spectrophotometer



Process by which gaseous atoms are produced in flames

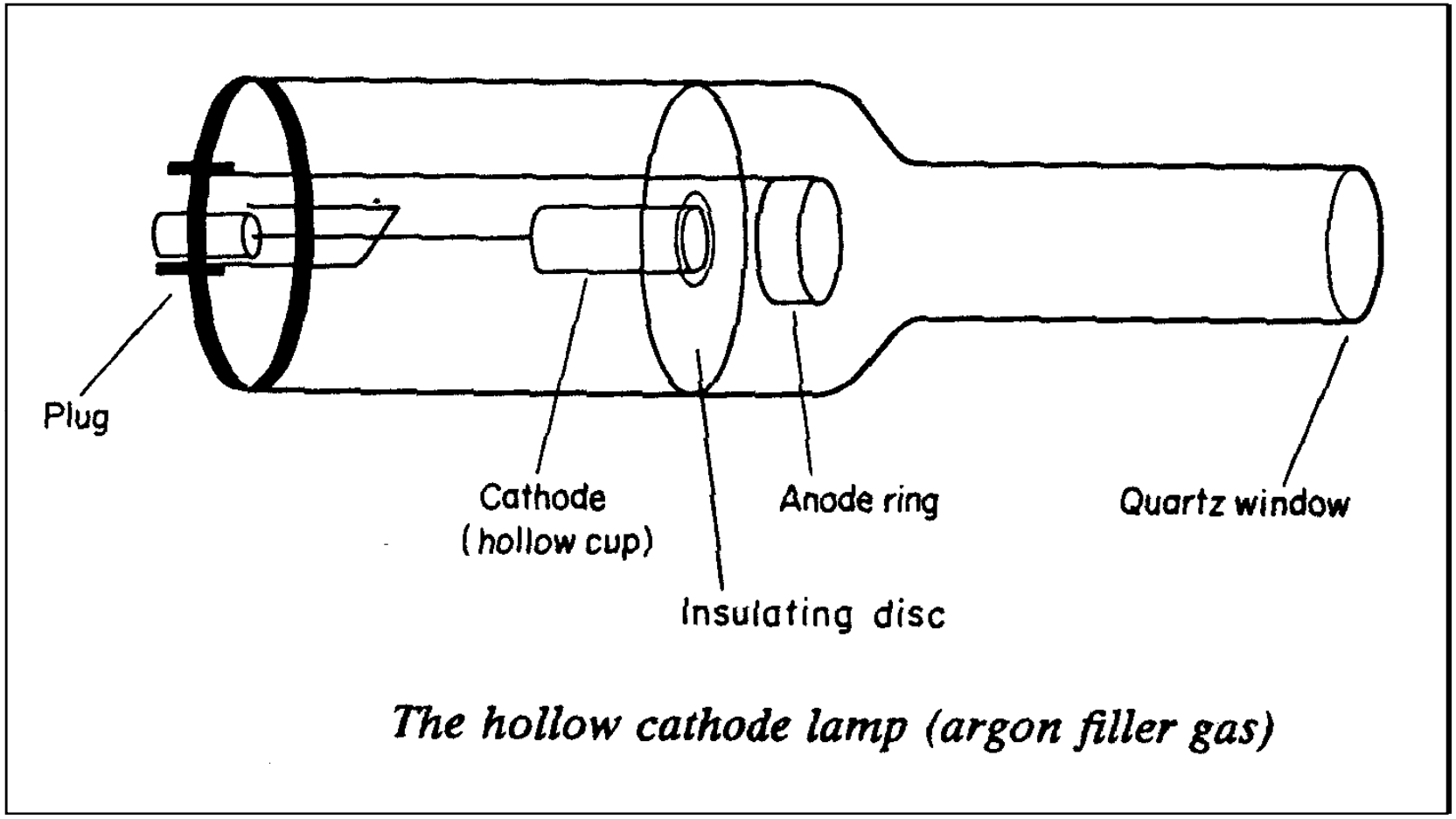
Radiation Source

- Beer's Law only applies to **monochromatic radiation.**
- In practice, **monochromatic** implies that the **linewidth** of the radiation being measured is less than the **bandwidth** of the absorbing species.
- Atomic absorption lines very sharp with an inherent linewidth of 0.0001 nm.
- Due to Doppler effect and pressure broadening, linewidths of atoms in a flame are typically 0.001 - 0.01 nm.
- Therefore, we require a source having a linewidth of less than 0.01 nm.
- Typical monochromator has a bandwidth of 1 nm i.e. x100 greater than the linewidth of the atom in a flame.

Hollow Cathode Lamp

- Used because of the requirement for a source of narrow lines of the correct frequency.
- **Hollow Cathode lamp**
  - filled with argon or neon at a pressure of 130 - 170 Pa (1 - 5 torr)

Hollow Cathode Lamp



The hollow cathode lamp (argon filler gas)

Calculations

- For an absorbance of 1.0 we require 1.0/0.00436 = 230 times the sensitivity.
- For Cu, sensitivity = 0.05 ppm
- For an absorbance of 1 we require a concentration of 11.5 ppm.
- Using scale expansion of 10 we can usually obtain an absorbance of 1 using a 1 ppm solution of copper.

Applications

- **Agricultural analysis**
  - soils
  - plants
- **Clinical and biochemistry**
  - whole blood, plasma and serum Ca, Mg, Li, Na, K, Cu, Zn, Fe etc.
- **Metallurgy**
  - ores, metals and alloys
- **Lubricating oils**
  - Ba, Ca, Mg and Zn additives
- **Greases**
  - Li, Na, Ca
- **Water and effluents**
  - many elements e.g. Ca, Mg, Fe, Si, Al, Ba
- **Food**
  - wide range of elements
- **Animal feedstuffs**
  - Mn, Fe, Co, Cu, Zn, Cr, Se
- **Medicines**
  - range of elements

Test your understanding

In atomic emission spectroscopy (AES), the lines in line spectra is

a. observed as light of a particular wavelength (colour)
b. observed as Infrared
c. Observed as Ultra violet
d. None of the above

In atomic absorption spectroscopy, the lines in spectrum is observed as

a. Microwave radiations are observed
b. white lines can be observed against a black background
c. black lines can be observed against a bright background
d. No lines are observed

The elemental composition of biological fluid can be easily measured by

a. NMR
b. Atomic emission spectroscopy
c. UV-Vis spectrophotometer
d. IR spectrophotometer

Suppose you have isolated one proteins and after purification you want to analyze the crystallinity and amorphous nature of protein. Which instrument you will choose in this scenario?

a. NMR
b. UV-VIS spectroscopy
c. X-ray diffraction spectroscopy
d. Irspectroscopy

References & Further reading

1. Wilson, K, Walker, J., Principles and Techniques of Practical Biochemistry. 5th Ed. - Cambridge University Press,. Cambridge 1999.
2. Biotechniques, Theory & Practice: Second Edition by SVS Rana, Rustogi Publications.
3. Biochemical Methods of Analysis, Saroj Dua And Neera Garg : Narosa Publishing House, New Delhi.
4. Bioanalytical Techniques, M.L. Srivastava, Narosa Publishing House, New Delhi.