BIO & PHARMA ANALYTICAL TECHNIQUES

Chapter 10
Metal & Mineral Analysis

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Chapter Description

- Aims
  - Discuss theory, principles and application of analytical techniques used in material characterisation, pre-formulation development, manufacturing process and storage stability.

- Expected Outcomes
  - Explain general facts of metal and mineral analysis including application in other field.
  - Illustrate theory and principle of both instruments: ICP-MS and TOC/AAS.
  - Discuss on the application of both instruments in pharmaceutical.

- References

  - Gunzler H. & Williams A. (2002). Handbook of Analytical Techniques. Wiley-VCH, Weinheim, Germany.
  - Mullertz, A., Perrie, Y. and Rades, T. (2016) Analytical Techniques in the Pharmaceutical Sciences (Advances in Delivery Science and Technology). Springer, United States.

HEAVY METALS

- In technical terms, every metal with a density greater than 5 g/cm^3 is considered to be a **heavy metal.**

- In common, “heavy metal” is typically understood to mean an element that is toxic.

HEAVY METALS: origin

- Heavy metals are largely found in nature as minerals and ores.
- They get into the environment as a result of being extracted, from erosion or from volcanic activity.

MEDICAL PLANTS :

Intake of heavy metals occurs in the growth process of plants by absorption from water, from the ground and by aerosols from the air.

Contamination can also occur from the spillage of pesticides or sewage sludge containing heavy metal.

Raw Materials & Finished Products

- In the manufacturing of pharmaceutical products, catalysts containing heavy metals are often involved in the synthesis.
- Heavy metals can also transfer into the process by abrasion or by leaching (e.g. Fe, Cd, Cu, Cr, Hg and so on.)
- If they are not removed efficiently, then the tainted products could get into the market.
- Traces of inorganic impurities can reduce drug stability and shelf life of some pharmaceutical products

LEGAL REGULATIONS

- For many years, it has been known that certain heavy metals exhibit toxic effects even at low concentrations.
- As a result, limit values for the protection of the patients have been defined in the legislation and in the various pharmacopoeias (e.g. Ph. Eur., USP, JP, BP).
- For years USP <231> Heavy Metals was used

CONVENTIONAL METHOD

USP<231> is a limit **test for heavy metals** in samples.

- It is a **qualitative** (at best semi-quantitative) test that indicates the content of metallic impurities by **colored sulfide precipitate**
- Elements for which the method can be used are silver, arsenic, bismuth, cadmium, copper, mercury, molybdenum, lead, antimony, and tin.

  - Colorimetric method
  - Sample ignition and ashing at 600°C
  - Addition of H2S (thioacetamide)

  -Visual (subjective) comparison of color of metal sulfide precipitates

Disadvantages of Heavy metal test (H_2S)

- Difficulties in reproducibility
  - Colour of solution is subjective,
    standards change with time;

- Difficulties with reagents – safety issues
  - All procedures generate H_2S (including Thioacetamide) and H_2S more toxic than cyanide
  - Thioacetamide is not allowed in several countries

- Non-discriminatory screening test
  - Not element specific
  - Sensitivity varies by element

Why is elemental analysis important for pharmaceutical products?

- Elemental analysis allows us to quantifiably measure the presence of impurities within the sample, whether it's the product, excipient or packaging.

- Contaminants can be toxic, cause side effects, or affect the shelf-life or drug stability.

DETECTION METHODS

- Inductively couple plasma (ICP-MS and ICP-OES) are fast multi-element techniques, capable of analysing as many as 70 elements in a 2-min run after sample digestion.
- These powerful techniques can identify and quantify each metallic impurity with higher sensitivity and selectivity than conventional precipitation-based detection methods.
- Detection limits down to ppb or even ppt levels.

ICP-MS (MASS SPECTROMETRY)

ICP-OES (OPTICAL EMISSION SPECTROMETRY)

INDUCTIVELY COUPLE PLASMA MASS SPECTROMETRY

- In ICP-MS, the ions generated in the inductively coupled plasma will be separated by an interface in the high vacuum section of the spectrometer according to their mass-to-charge ratio.

Source: https://en.wikipedia.org

Why is ICP-MS unique?

ICP-MS can:

- measure almost any element at ppt to ppm levels in almost any material.
- measure all elements in a single analysis.
- distinguish different element species (speciation).

Main requirements in pharmaceutical analysis are:

- high sensitivity
- good matrix tolerance
- low levels of interferences
- ease of coupling to speciation techniques

PRINCIPLE

- Liquid samples to form aerosol in nebulizer.
- Introduction of Argon to the ICP torch, which is located in center of a radio frequency (RF) coil for energy supply.
- RF field causes collisions of Ar atoms, generating a high-energy plasma.
- Sample aerosol decomposed in plasma (6000 - 10000 K) to form analyte atoms which are simultaneously ionized.
- Ions extracted from the plasma into mass spectrometer region.

ICP-MS: PROCESS

Sample Introduction

ICP Torch

Interface

Mass Spectrometry

SAMPLE INTRODUCTION

- Methods depend on the physical characteristics
- **Liquids:**

  Nebulizer

  Sample is pump out from the vial into the nebulizer.

  (add argon gas → small amount of aerosol)

- **Solids:**

  Electrothermal vaporizer / Laser

  Electric current to rapidly vaporize a solid sample

- Samples must be introduced to the ICP torch in either a gaseous/aerosol form

ICP TORCH

- ICP torch consists of a copper induction coil wrapped around a concentric quartz structure
- Argon gas is continuously flowing throughout the quartz structure
- Reaction between argon gas and the copper induction coil creating very high temperatures → plasma
- Constant temperature around 8000°C during the analysis

- Aerosol → enters high temp torch → plasma (atomization) →atoms

- Atoms travel through the plasma → absorbing energy until release an electron (ionization) → ions

↓

Newly formed ions travel out of the torch to the interface

INTERFACE

- Point where samples from the ICP portion of the instrument is introduced to the MS portion of the instrument.
- The interface portion serves to allow the ICP and the MS portions to be coupled.

- i. Sampler cone : water cooled cone with a small orifice (rapid cooling of the gas)

- ii. Skimmer cone : a fraction of this gas passes through a skimmer cone and into a chamber that is maintained at a vacuum of the MS (atmospheric → vacuum)

- This is to ensure the ionic gas enter the MS at proper temperature and pressure

MASS SPECTROMETRY

Ion stream is focused into the MS by single ion lenses (ions generated in plasma are nearly all positively charged and hv tendency to repel each other)

↓

Ions pass through a charged metallic cylinder which keeps the ions from diverging

↓

Ions then dispersed in the mass analyzer, detected by the detector based on their mass-to-charge ratio

ADVANTAGES

- Wide elemental coverage
- Very low detection limits(ppt /ppm)
- Fast analysis times (all elements at once)
- Simple spectra
- Isotopic information
- measure almost any element at ppt to ppm levels in almost any material.
- measure all elements in a single analysis.
- distinguish different element species (speciation).

INTRODUCTION:

ATOMIC ABSORPTION SPECTROSCOPY (AAS)

- Atomic Absorption Spectroscopy is a very common technique for detecting metals and metalloids in samples.
- It is very reliable and simple to use.
- It can analyze over 62 elements.
- It also measures the concentration of metals in the sample.

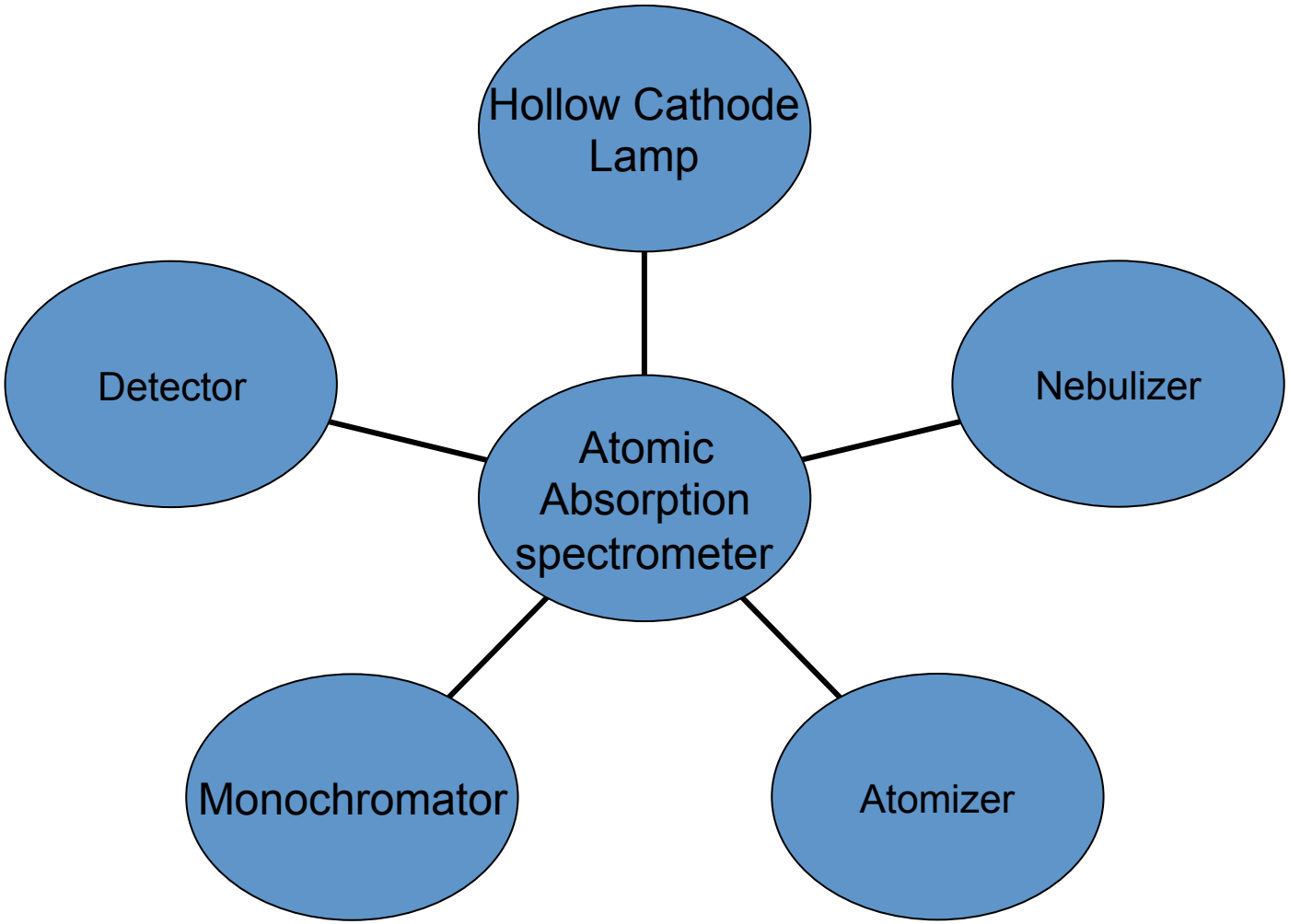
PRINCIPLE OF AAS:

- The technique uses basically the principle that free atoms (gas) generated in an atomizer can absorb radiation at specific frequency.
- Atomic-absorption spectroscopy quantifies the absorption of ground state atoms in the gaseous state .
- The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels.
- The analyte concentration is determined from the amount of absorption.

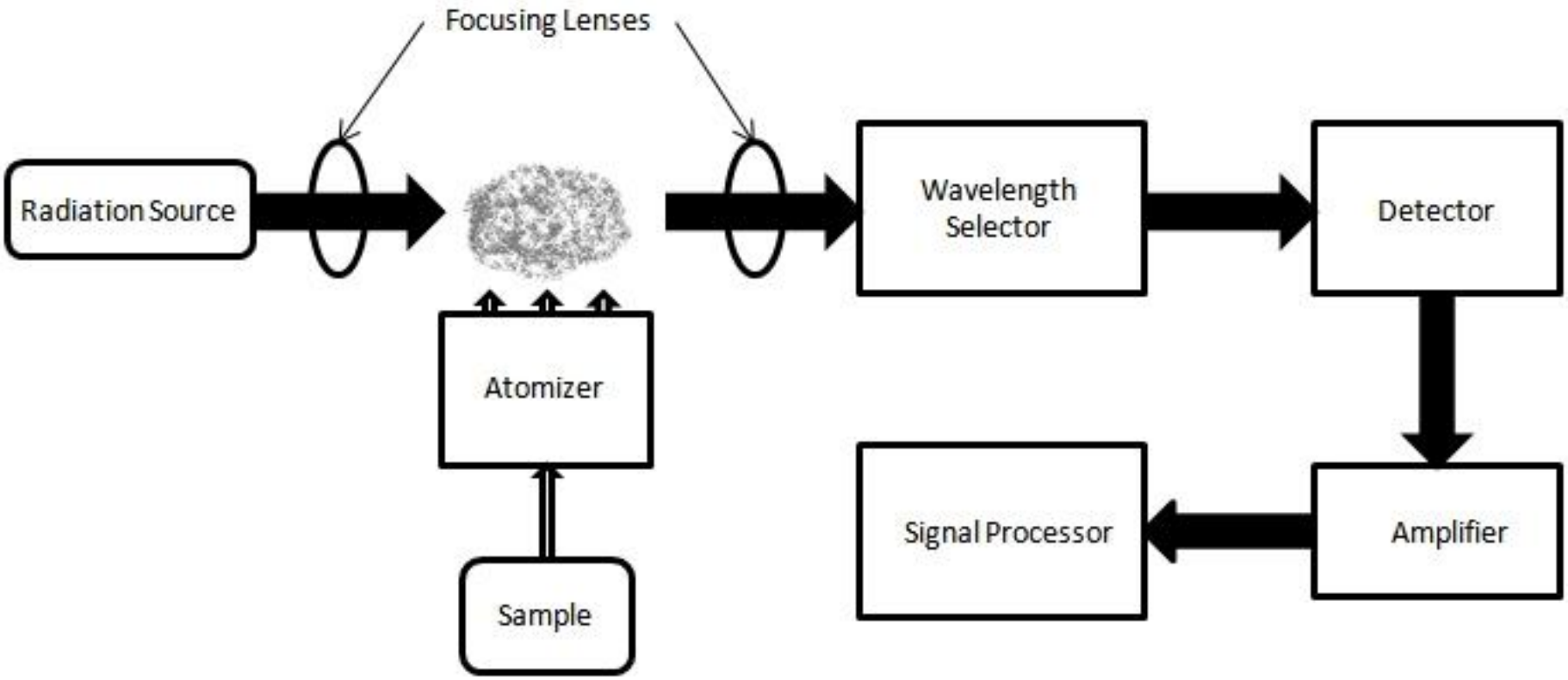
- Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration.

- Atomic absorption is a very common technique for detecting metals and metalloids in environmental samples.

THEORY:



Schematic diagram of AAS:

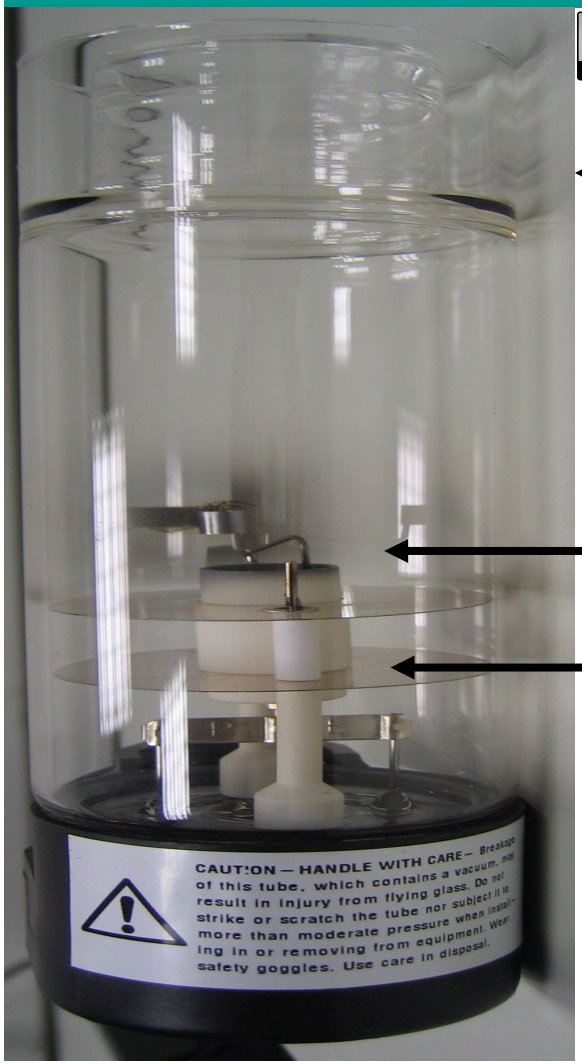


Source:https://commons.wikimedia.org

LIGHT SOURCE

- **Hollow Cathode Lamp** are the most common radiation source in AAS.
- It contains a **tungsten anode** and a hollow cylindrical cathode made of the element to be determined.
- These are **sealed in a glass tube** filled with an inert gas (neon or argon) .
- Each element has its own unique lamp which must be used for that analysis .

HOLLOW CATHODE LAMP



Quartz window

Anode

Cathode

NEBULIZER

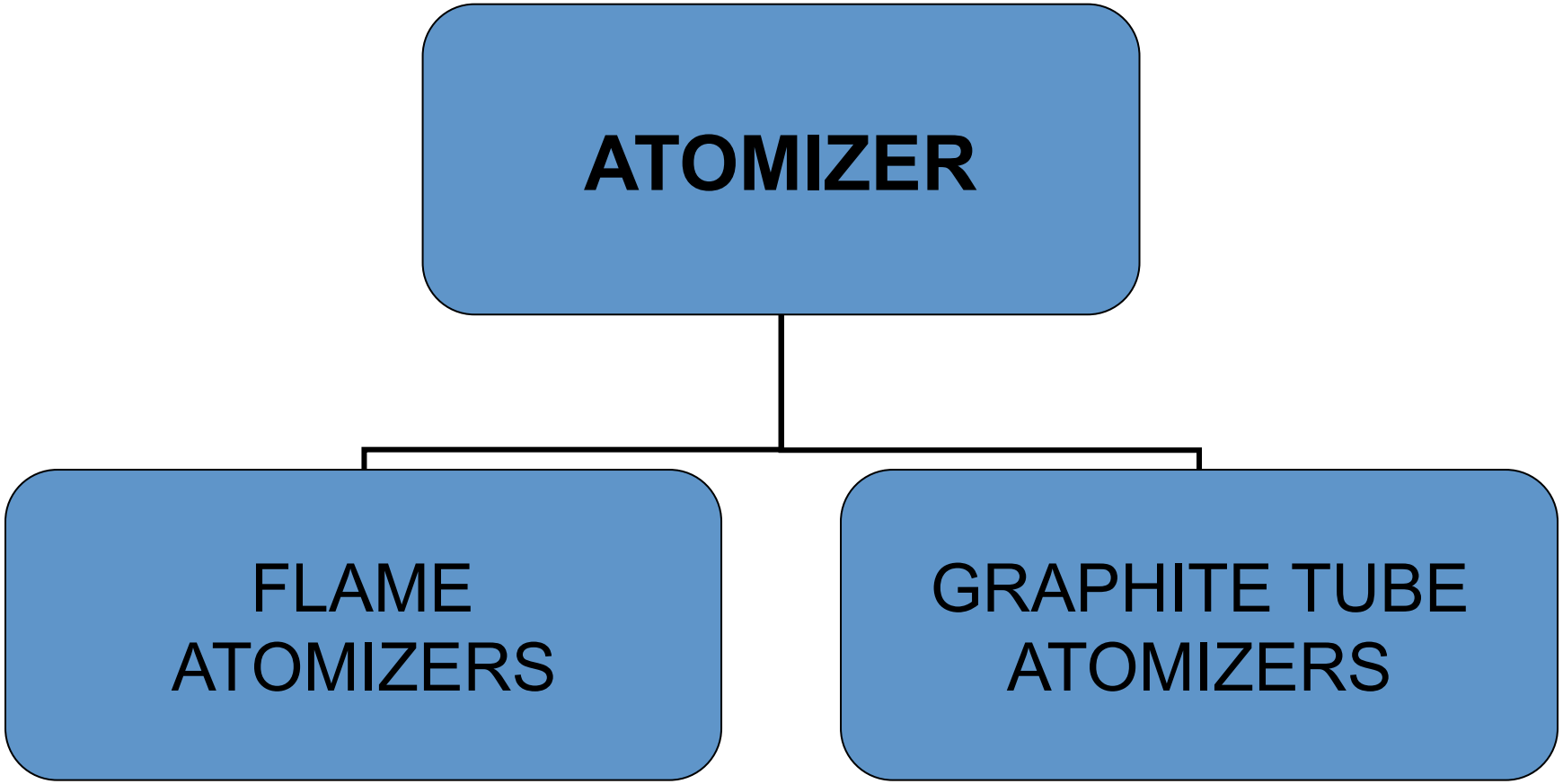
- suck up liquid samples at controlled rate.
- create **a fine aerosol spray** for introduction into flame.
- Mix the aerosol and fuel and oxidant thoroughly for introduction into flame.

ATOMIZER

- **Elements** to be analyzed needs to be in **atomic state.**

- **Atomization** is **separation of particles into individual molecules and breaking molecules into atoms**. This is done by exposing the analyte to high temperatures in a flame or graphite furnace .

ATOMIZERS:



FLAME ATOMIZER:

- To **create flame**, we need to **mix an oxidant gas and a fuel gas.**
- In most of the cases air-acetylene flame or nitrous oxide-acetylene flame is used.
- **Liquid or dissolved samples** are typically used with flame atomizer.

GRAPHITE TUBE ATOMIZER:

- Uses a **graphite coated furnace** to vaporize the sample.
- In GFAAS sample, **samples are deposited in a small graphite coated tube** which can then be heated to vaporize and atomize the analyte.
- The graphite tubes are **heated** using a high current power supply.

MONOCHROMATOR

- This is a very important part in an AA spectrometer. It is used **to separate out all of the thousands of lines.**

- A monochromator is used to **select the specific wavelength of light which is absorbed by the sample**, and to exclude other wavelengths.

- The selection of the specific light allows the determination of the **selected element** in the presence of others.

DETECTOR

- The light selected by the monochromator is directed onto a detector that is typically a **photomultiplier tube**, whose function is to **convert the light signal into an electrical signal** proportional to the light intensity.

- The processing of electrical signal is fulfilled by a signal amplifier.

- The signal could be displayed for readout, or further fed into a data station for printout by the requested format.

APPLICATION IN PHARMA

- **Drug discovery / drug development**:

  Target element analysis

  Simple metal analysis during development of metal-based Drugs

- **QA/QC and process development**:

  National Pharmacopeia (e.g. USP, EP, JP) Testing Impurity limit tests

  Metals in active pharmaceutical ingredients (API,exipients)

  QC of natural products – toxic impurities

- **Pharmaceutical waste water**

Conclusion of the Chapter

ICP-MS and AAS are the new Gold Standard for metals testing in pharmaceutical materials.

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Any Question?

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