2.3.Atomic Absorption Spectrometry(AAS) & Chromatography

Outline:

- Introduction
- Working principle of AAS
- Basic component of AAS
- Application of AAS
- Definition of Chromatography
- Components of Chromatography
- Application of Chromatography
- Types of Chromatography

INTRODUCTION

Objectives:

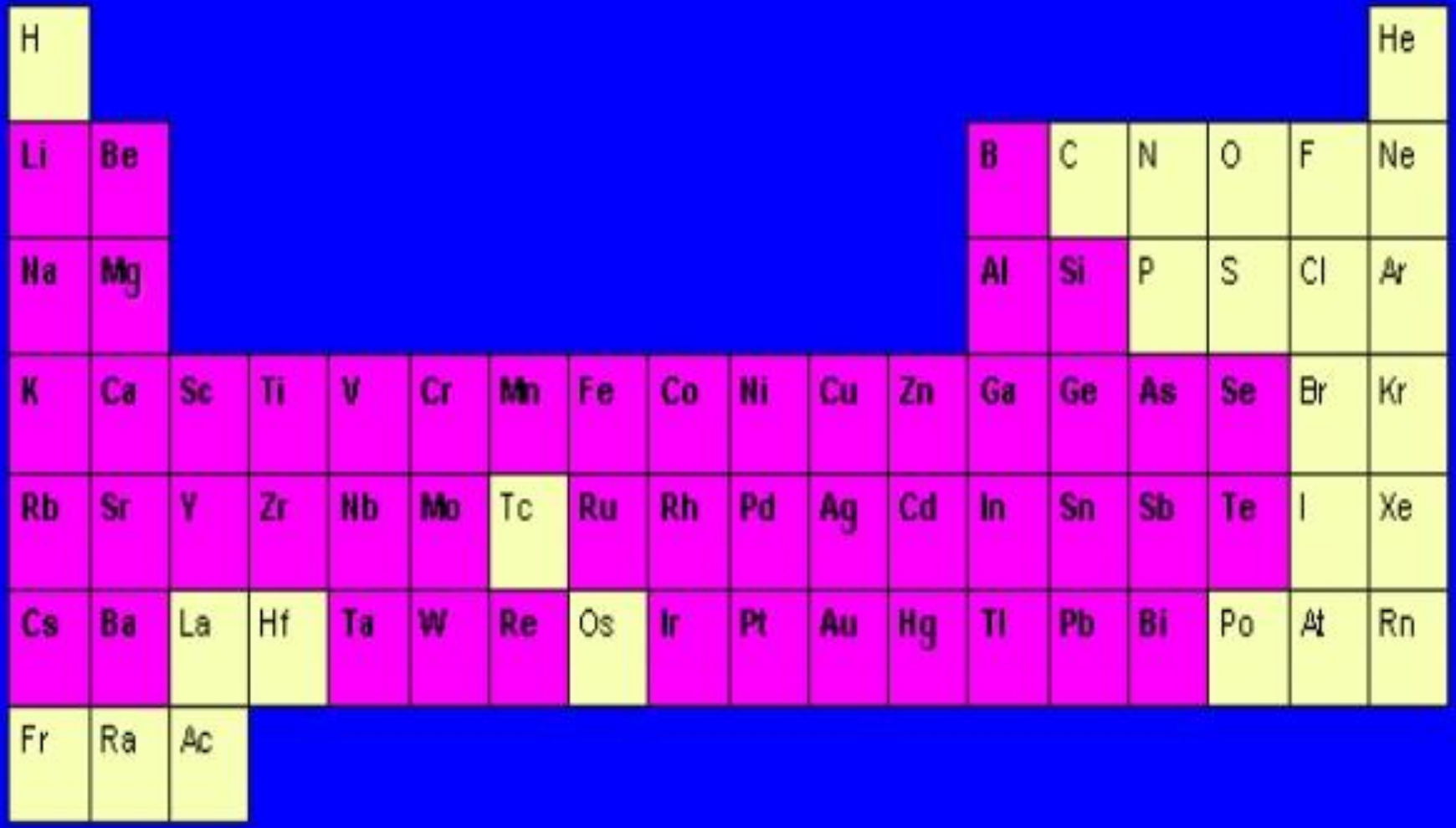
- By the end of the session students will explain:
  - working principle & application of AAS
  - Application and type of chromatography

CONT'D…

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

Cont'd…

Elements detectable by atomic absorption are highlighted in pink in this periodic table



Cont'd…

ACTIVITY -1

- What is the work principle of atomic absorption spectroscopy?

3min.

Cont'd…

❑Working Principle of AAS:

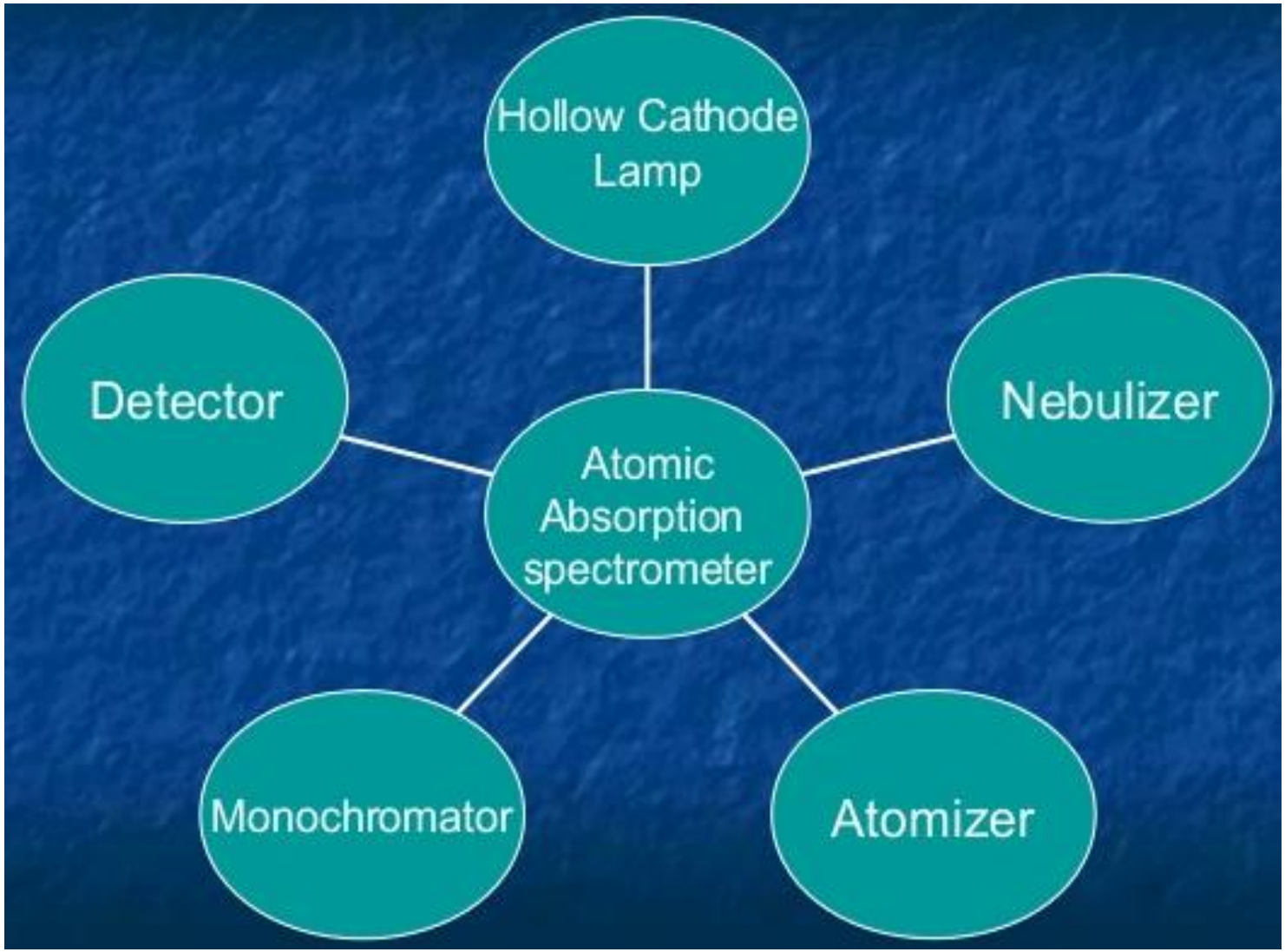
- The techniques basically the principle that free atoms(gas) generated in an atomizer can absorb radiation at specific frequency.
- Atomic absorption spectroscopy quantifies the absorption of ground state atoms in the gaseous state.
- The atoms absorb ultraviolet or visible light and make transitions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

Cont'd…

- 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 in environmental samples.

Cont'd…

Basic components of AAS are:



AAS:

Cont'd…

Cont'd…

ACTIVITY -2

- What are the function of five basic components of AAS?

6min.

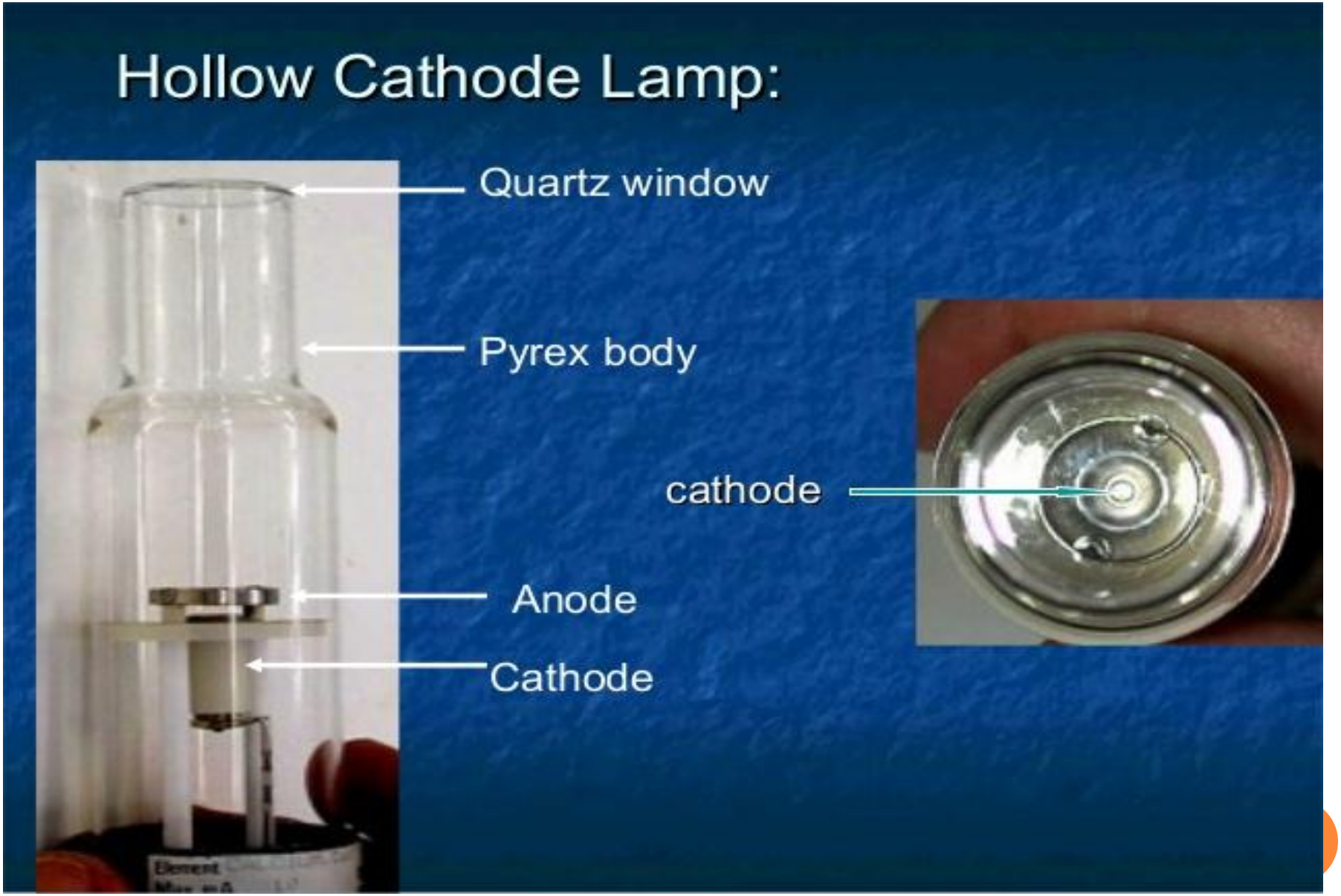
Cont'd…

❑Basic components of AAS are :

1. Hollow Cathode Lamp are the most common radiation source or light 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.

Cont'd…


Hollow Cathode Lamp:
Quartz window
Pyrex body
cathode
Anode
Cathode

Cont'd…

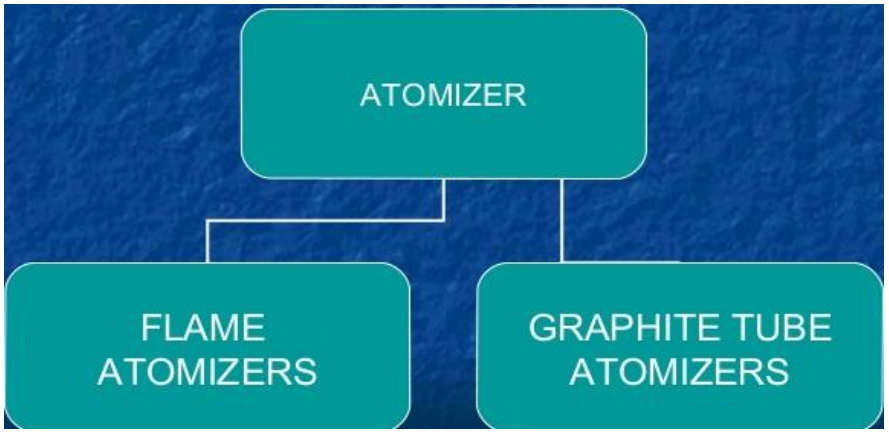
2. Nebulizer:

- Suck up liquid samples at controlled rate.
- Create a fine aerosol spray for introduction into flame.
- Mix the aerosol and fuel and oxidant systematically for introduction into flame.

3.ATOMIZER:

Cont'd…

- **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.

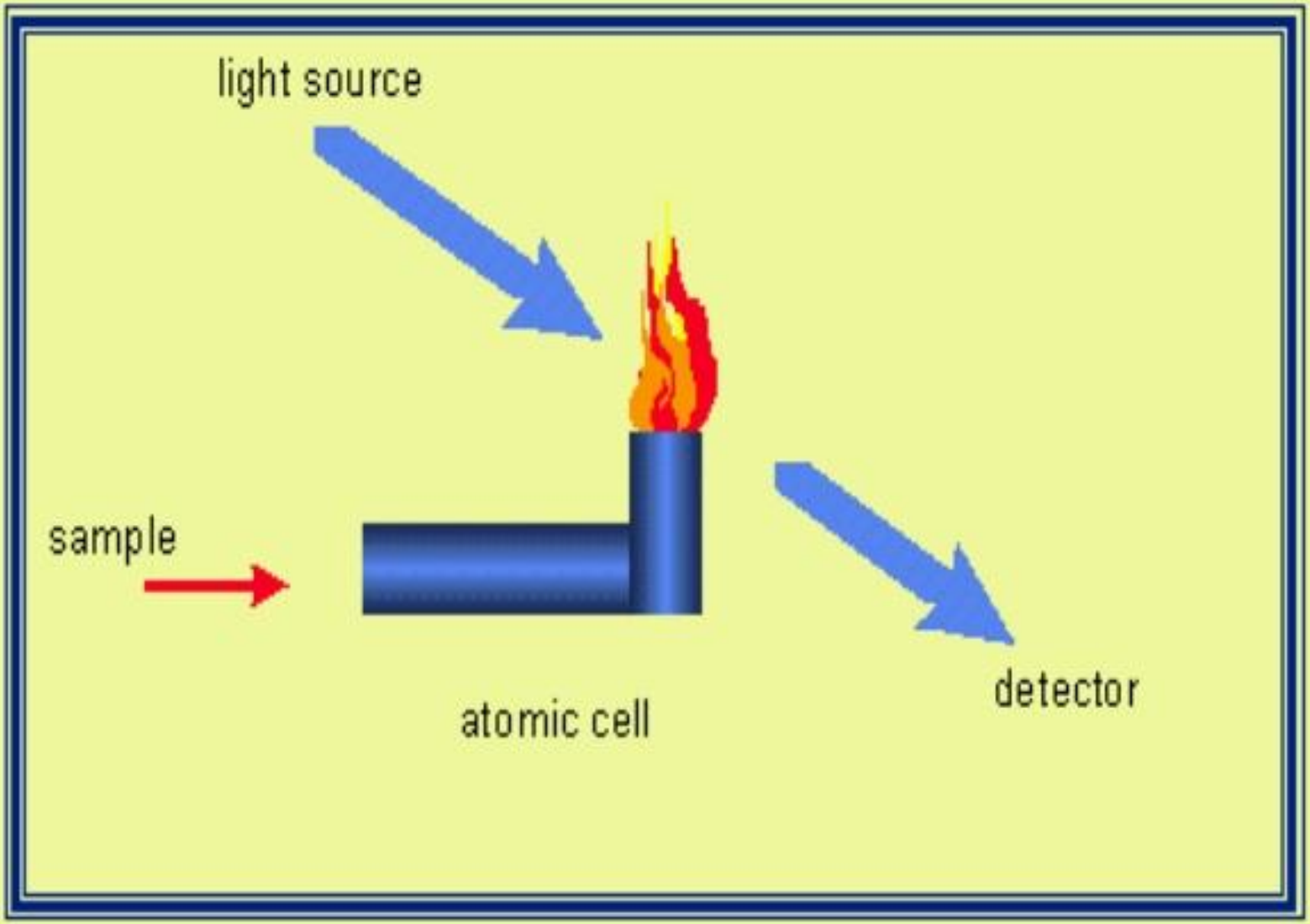


Cont'd…

❑ 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.

Cont'd…



Cont'd…

❑GRAPHITE TUBE ATOMIZER:

- Uses a graphite coated furnace to vaporize the 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.

Cont'd…

4. 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.

Cont'd…

5. 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 read out,or further fed into a data station for printout by the requested format.

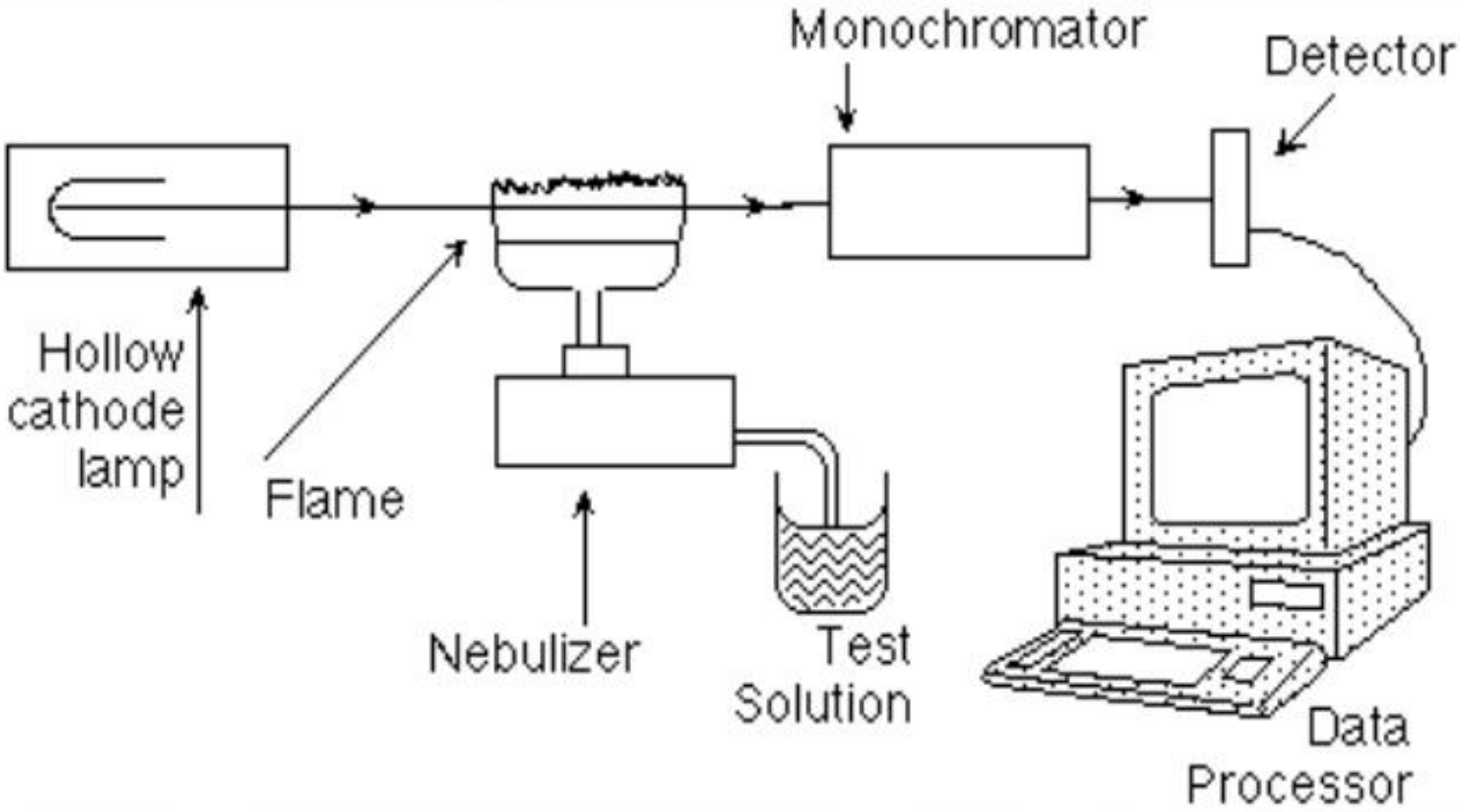
Cont'd…

❑CALIBRATION CURVE:

- A calibration curve is used to determine the unknown concentration of an element in a solution.
- The instrument is calibrated using several solutions of known concentrations.
- The absorbance of each known solution is measured and then a calibration curve of concentration vs absorbance is plotted.
- The sample solution is fed into the instrument, and the absorbance of the element in this solution is measured.
- The unknown concentration of the element is then calculated from the calibration curve.

Cont'd…

Schematic diagram of AAS:



ACTIVITY-3

- What are the application of AAS?

3min.

Cont'd…

❑APPLICATIONS OF AAS ARE:

- ❖ Determination of even small amounts of metals(lead, mercury,calcium,magnesium,etc) as follows:
- ✓ Environmental studies: drinking water, ocean water & soil
- ✓ Food industry
- ✓ Pharmacy industry.

2.4.CHROMATOGRAPHY

❑ CHROMATOGRAPHY:

- It is a technique used to separate and identify the components of a mixture.
- Works by allowing the molecules present in the mixture to distribute themselves between a stationary and a mobile medium.
- Molecules that spend most of their time in the mobile phase are carried along faster.

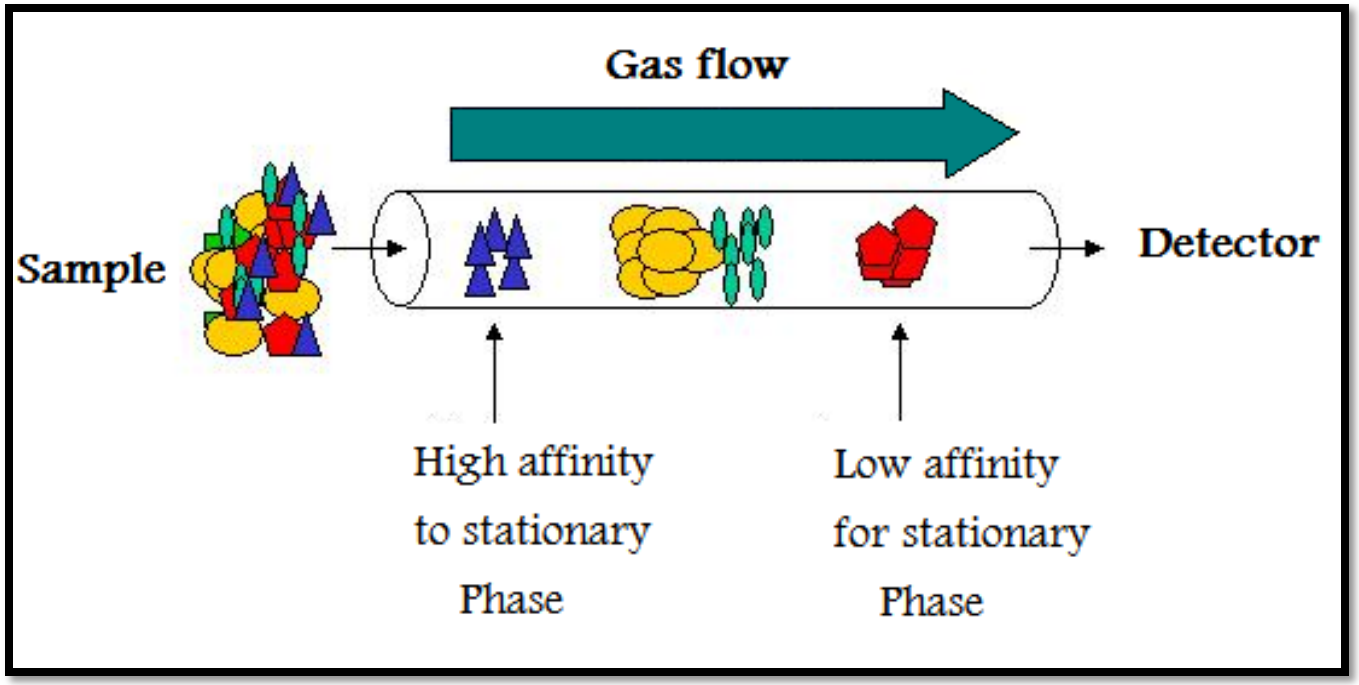
Introductory Principles

Chromatography is a combination of two words;

* **Chromo** – Meaning color

* **Graphy** – writing/representation of something on paper/

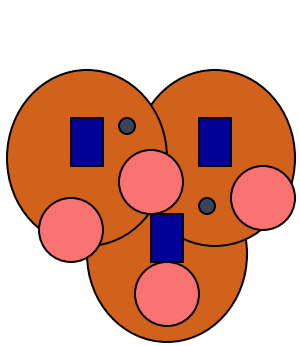
Principle: the samples are subjected to flow by mobile liquid onto or through the stable stationary phase.



DEFINITION

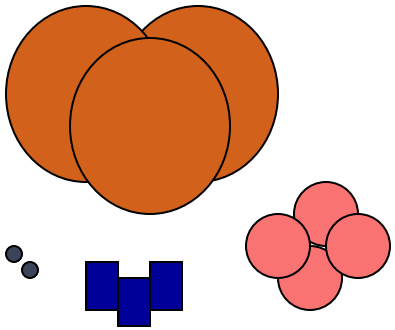
- Chromatography is a physical separation method of separation in which the components of a mixture are separated by differences in their distribution between two phases,
- One of which is stationary (**stationary phase**)
- while the other (mobile phase) moves through it in a definite direction.
- The substances must interact with the stationary phase to be retained and separated by it.

CHROMATOGRAPHY



Mixture

Separate



Components

- Analyze
- Identify
- Purify
- Quantify

ACTIVITY-4

- **What are the components of chromatography?**

3min.

Components of Chromatography are:

- **Mobile Phase** – gas or liquid that carries the mixture of components through the stationary phase.
- **Stationary Phase** – the part of the apparatus that holds the components as they move through it, separating them. `

✓ The stationary phase may be a solid, or a liquid supported on a solid or gel.

Uses for Chromatography

Chromatography is used by scientists to:

- Analyze – examine a mixture, its components, and their relations to one another
- Identify – determine the identity of a mixture or components based on known components
- Purify – separate components in order to isolate one of interest for further study
- Quantify – determine the amount of the a mixture and/or the components present in the sample

Application for Chromatography

- ✓Pharmacy Company
- ✓Hospital
- ✓Law Enforcement
- ✓Environmental Agency
- ✓Food Manufacturing Plant

CHROMATOGRAPHY TERMS

Chromatogram:

It is the visual output of the chromatograph.

Chromatograph:

It is equipment that enables a sophisticated Separation.

Stationary phase (bounded phase):

It is a phase that is covalently bonded to the support particles or to the inside wall of the column tubing.

CHROMATOGRAPHY TERMS

Mobile phase:

It is the phase which moves in a definite direction.

Analyte (Sample):

It is the substance to be separated during chromatography.

Eluate:

It is the mobile phase leaving the column.

CHROMATOGRAPHY TERMS

Retention time:

It is the characteristic time it takes for a particular analyte to pass through the system (from the column inlet to the detector) under set conditions.

Eluent:

It is the solvent that will carry the analyte.

CHROMATOGRAPHY TERMS

Retardation factor (R):

Fraction of an analyte in the mobile phase of a chromatographic system.

$$R = \frac{Quantity\ of\ substance\ in\ mobile\ phase}{Total\ quantity\ of\ substance\ in\ the\ system}$$

ACTIVITY-5

- What are the types of chromatography?

4min.

TYPES OF CHROMATOGRAPHY

- **Liquid Chromatography** – separates liquid samples with a liquid solvent (mobile phase) and a column composed of solid beads (stationary phase)

- **Gas Chromatography** – separates vaporized samples with a carrier gas (mobile phase) and a column composed of a liquid or of solid beads (stationary phase)

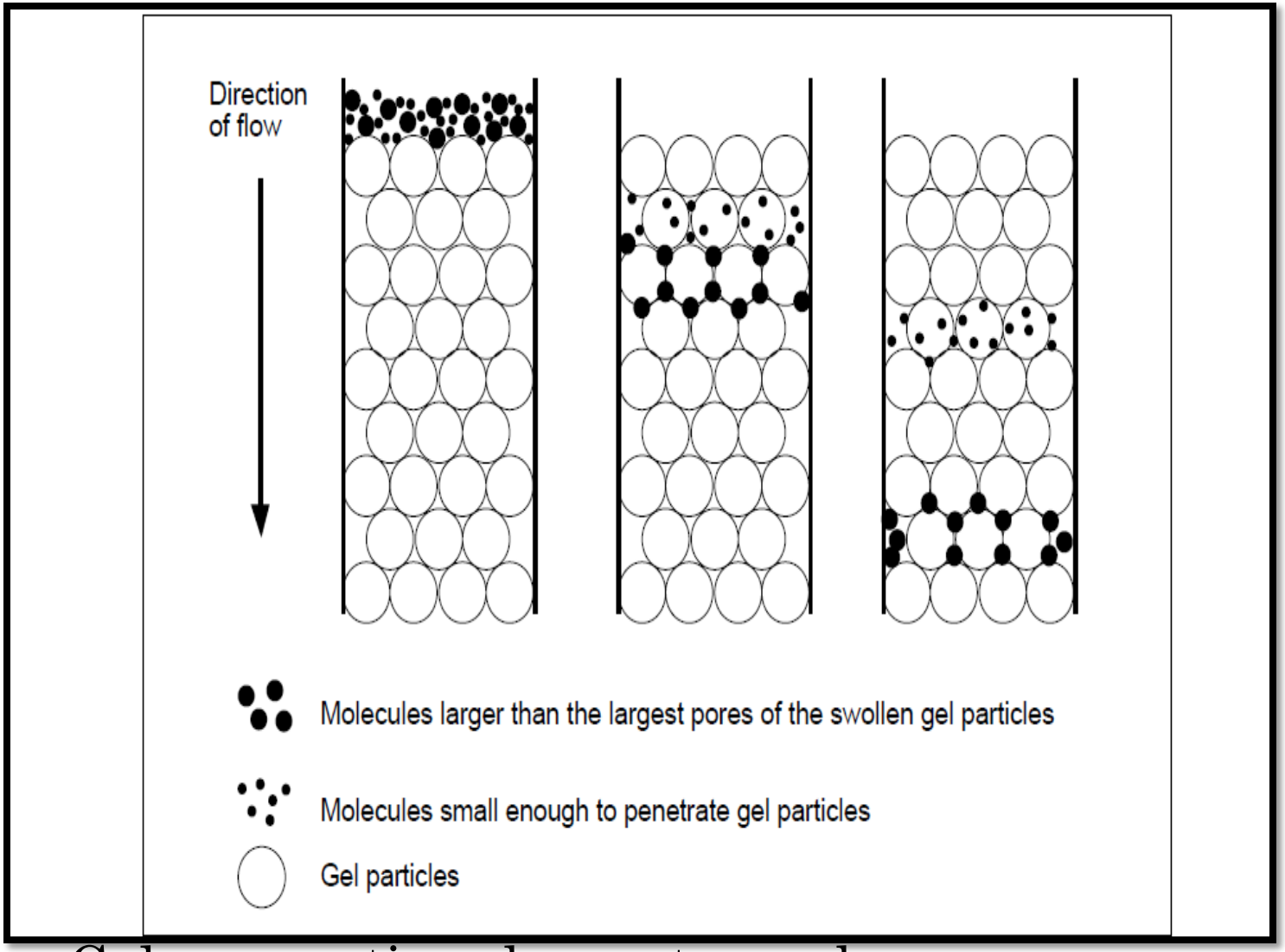
- **Paper Chromatography** – separates dried liquid samples with a liquid solvent (mobile phase) and a paper strip (stationary phase)

- **Thin-Layer Chromatography** – separates dried liquid samples with a liquid solvent (mobile phase) and a glass plate covered with a thin layer of alumina or silica gel (stationary phase)

GEL FILTRATION

- Gel filtration separates molecules according to the differences in size as they pass through the filtration medium packed in the column.
- It is well suited for biomolecules that are sensitive to pH ,concentration and harsh environment.
- Parameters that affects gel filtration are, particle size, flow rate, packaging density, porosity of the particle and viscosity of the mobile phase.

CONT'D.....



Gel permeation chromatography

MATERIALS REQUIRED ARE :

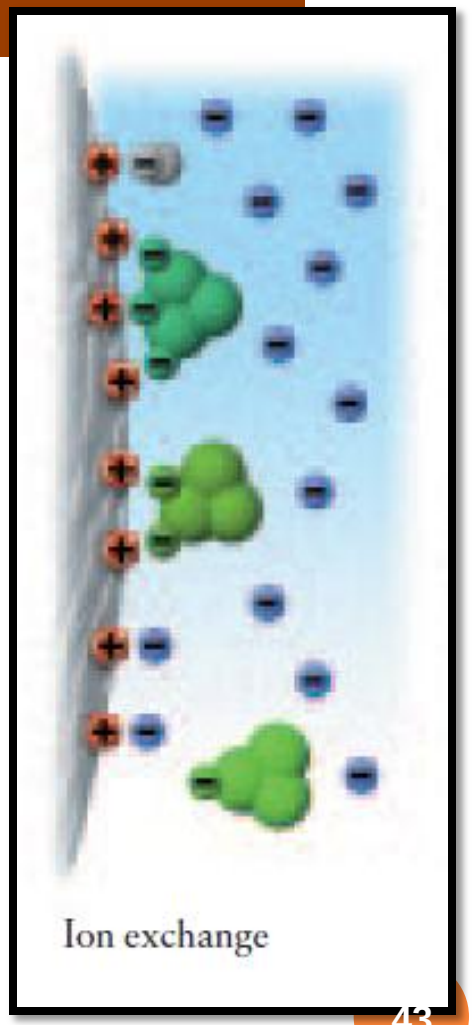
- ✓ Cross linked dextrans (sephadex)
- ✓ Agarose (sepharose)
- ✓ Polymer /Polyacrylamide/
- ✓ Porous glass gel.

APPLICATIONS

- ✓ Fractionation (purification of the desired protein using suitable gel)
- ✓ Molecular weight determination

ION EXCHANGE

- Ion exchange chromatography is used to **remove ions** of one type from a mixture and replace them by ions of another type.
- The basic principle is reversible competitive binding



ION EXCHANGERS

- Cation exchangers (negative ions – stationary)
- Anion exchangers (positive ions - stationary)
- Four types of polymers are commonly used. They are,
  - ✓ Synthetic hydrophobic polymer resins cross linked with divinely benzene.
  - ✓ Naturally occurring as well as synthetic polymers(cellulose)
  - ✓ Synthetic hydrophilic polymers
  - ✓ Silica gel

AFFINITY CHROMATOGRAPHY:

- Affinity chromatography includes bioaffinity, dye-ligand affinity and immobilized metal ion afffinity techniques.
- It is based on the formation of the specific and reversible complexes between a pair of biomolecules.

Affinity

ACTIVITY-6

- Summarized the lesson

THANK YOU