UNIT II

Chromatography – Principle, theory, instrumentation and applications in chemical analysis of the following – column, paper, thin layer and ion-exchange – GC, GLC and HPLC. Purification of common organic solvents.

Atomic absorption spectroscopy and Flame emission spectroscopy – basic principle – Instrumentation and applications. Comparison between AAS and FES.

REFERENCES

1. Willard, Merrit and Dean, Instrumental Methods of Chemical Analysis
2. Chatwal, Instrumental Methods of Analysis
3. Sharma, Instrumental Methods of Chemical Analysis
4. Kenner, Analytical Separations and Determinations
5. Sharma, Chromatography

PRINCIPLES AND APPLICATION OF CHROMATOGRAPHY

CHROMATOGRAPHY

- Laboratory technique for the Separation of mixtures
- *Chroma* -"color" and *graphein* - "to write".
- Colour bands - separation of individual compounds
- Measured or analysed.

PURPOSE OF CHROMATOGRAPHY

- **Analytical**

✓ Determine Chemical composition of a sample

- **Preparative**

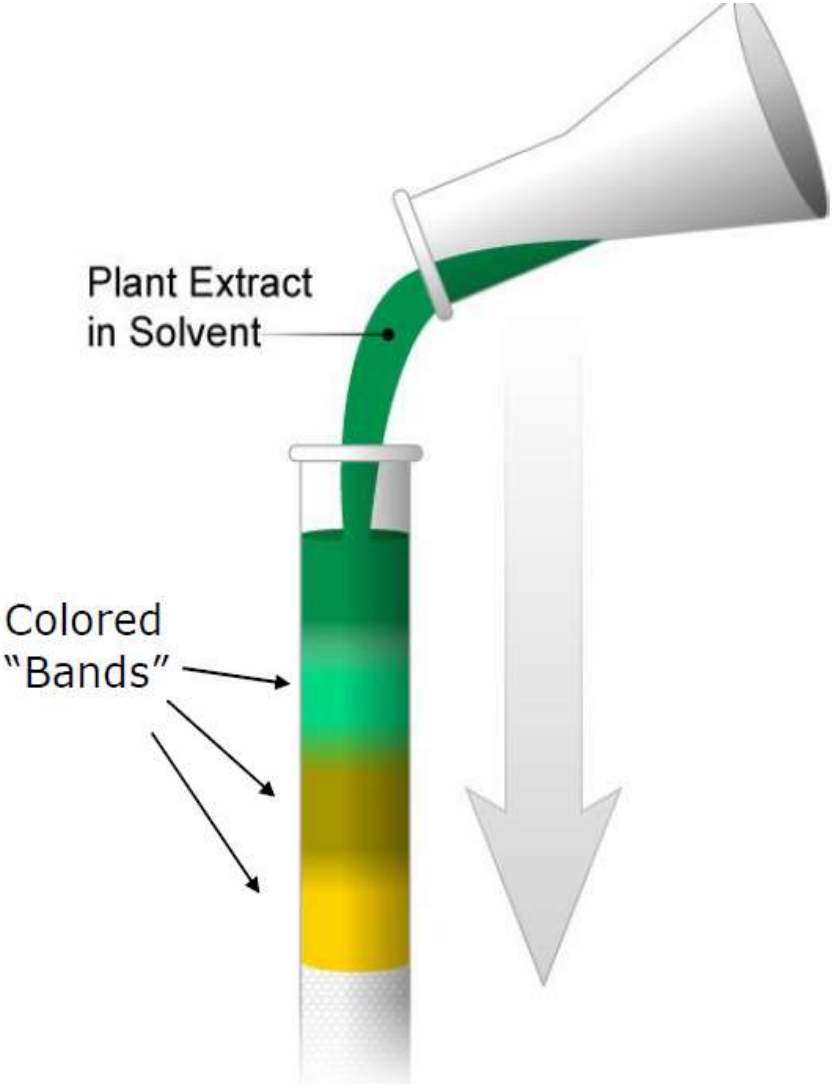
✓ Used to purify sufficient quantities of a substance

TSWETT EXPERIMENT

- Tall glass open column filled with sand-like particles
- Ground-up plant extract
- Poured into the column and saw colored "bands" develop as the extract percolated down thru the column
- Different compounds had separated

Greek

Chroma -- color
Graphy -- writing/study of



CHROMATOGRAPHY TERMS

- **Chromatograph** - equipment that enables a sophisticated separation

  EX. Gas chromatography or Liquid chromatography
- **Eluent** - Fluid entering column/ solvent that carries the analyte.
- **Eluate** - Mobile phase leaving the column.
- **Stationary phase - Immobilized phase**

❖ Immobilized on the support particles or on the inner wall of the column tubing.

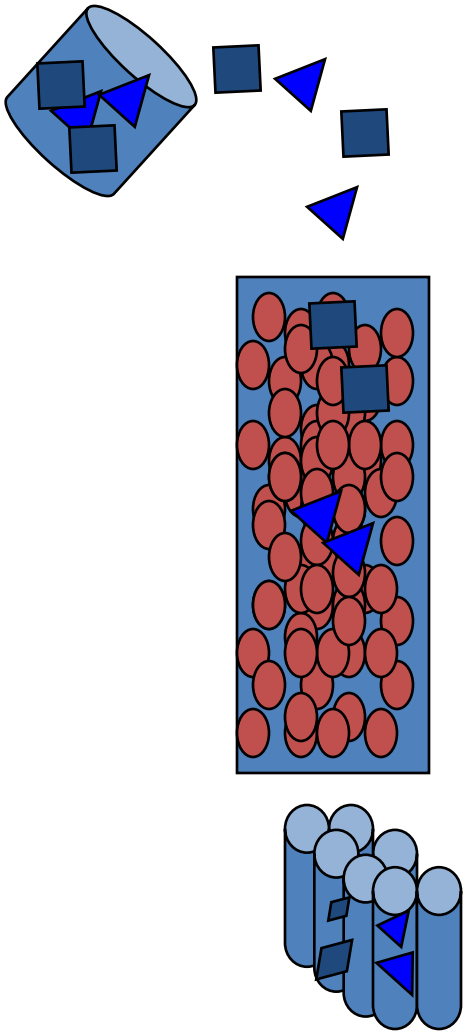
❖ Examples : Silica layer - Thin Layer Chromatography

- **Mobile phase**

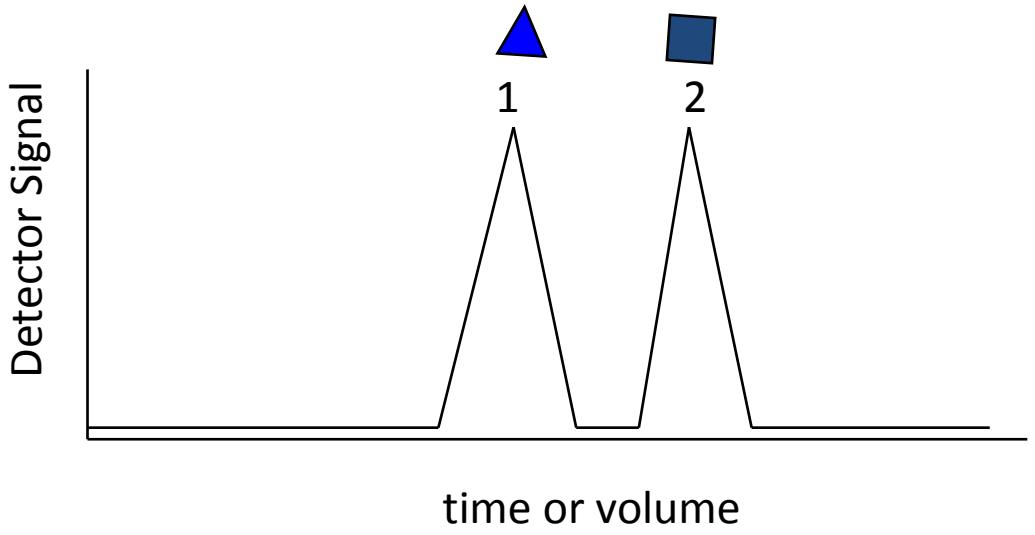
  Moves in a definite direction. Liquid (LC), Gas (GC).
- The mobile phase moves through the chromatography column (the stationary phase) where the sample interacts with the stationary phase and is separated.
- **Retention time :** Time takes for a particular analyte to pass through the system (from the column inlet to the detector) under set conditions.
- **Sample (Anylate)** :Substance analyzed in chromatography.
- **Solvent** : Any substance capable of solubilizing another substance.

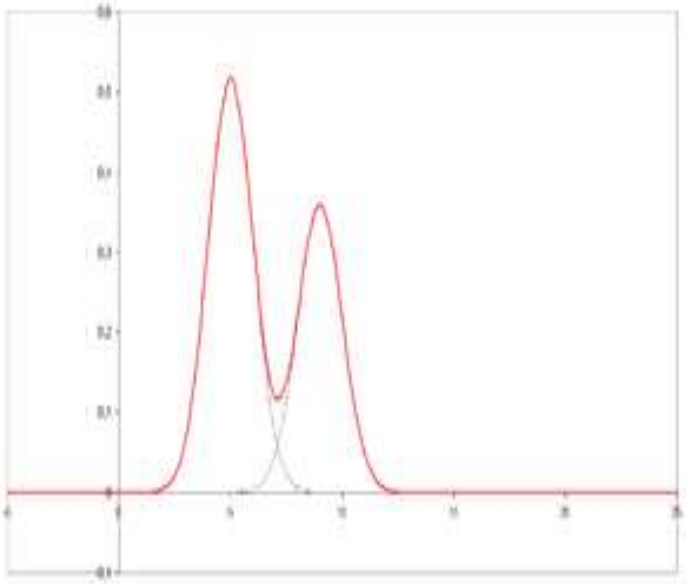
❑ Chromatogram

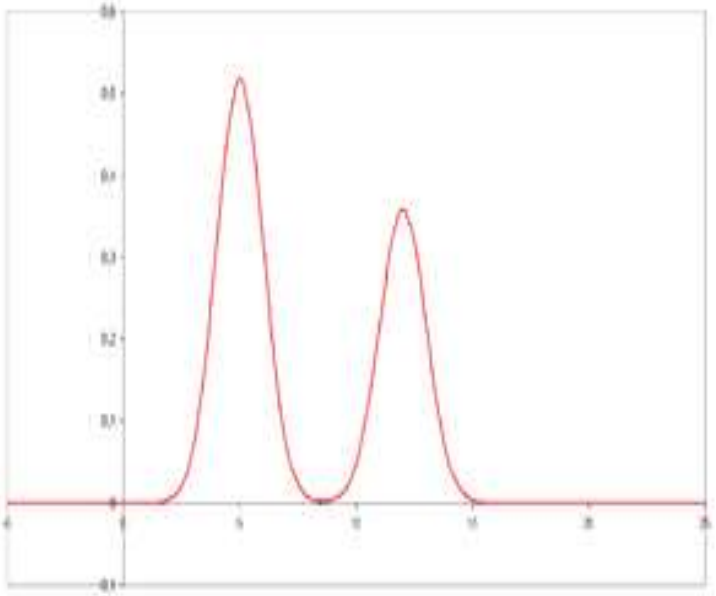
- Visual output of the chromatograph.
- Separation - Different peaks or patterns on the chromatogram correspond to different components of the separated mixture.



Chromatogram - Detector signal vs. retention time or volume







- X- axis - Retention time
- Y-axis - Signal
- Signal is proportional to the concentration of the specific analyte separated.

HOW TO DESCRIBE A CHROMATOGRAM

Chromatogram - response of a detector vs time. - shows when various components come off a column

Retention time t_r - The time at which a component elutes from a column.

Theoretical Plates

Assume a chromatographic peak has a Gaussian shape

H is height of peak

$w_{1/2}$ is with at ½ height

(If true Gaussian $w_{1/2} = 2.35\sigma$

Where σ= standard deviation)

Width at baseline should be 4σ

For chromatography we retain the name Theoretical Plate, but calculate it using the retention time and the width at ½ height

$$N = \frac{5.5 t_r^2}{w_{1/2}^2}$$

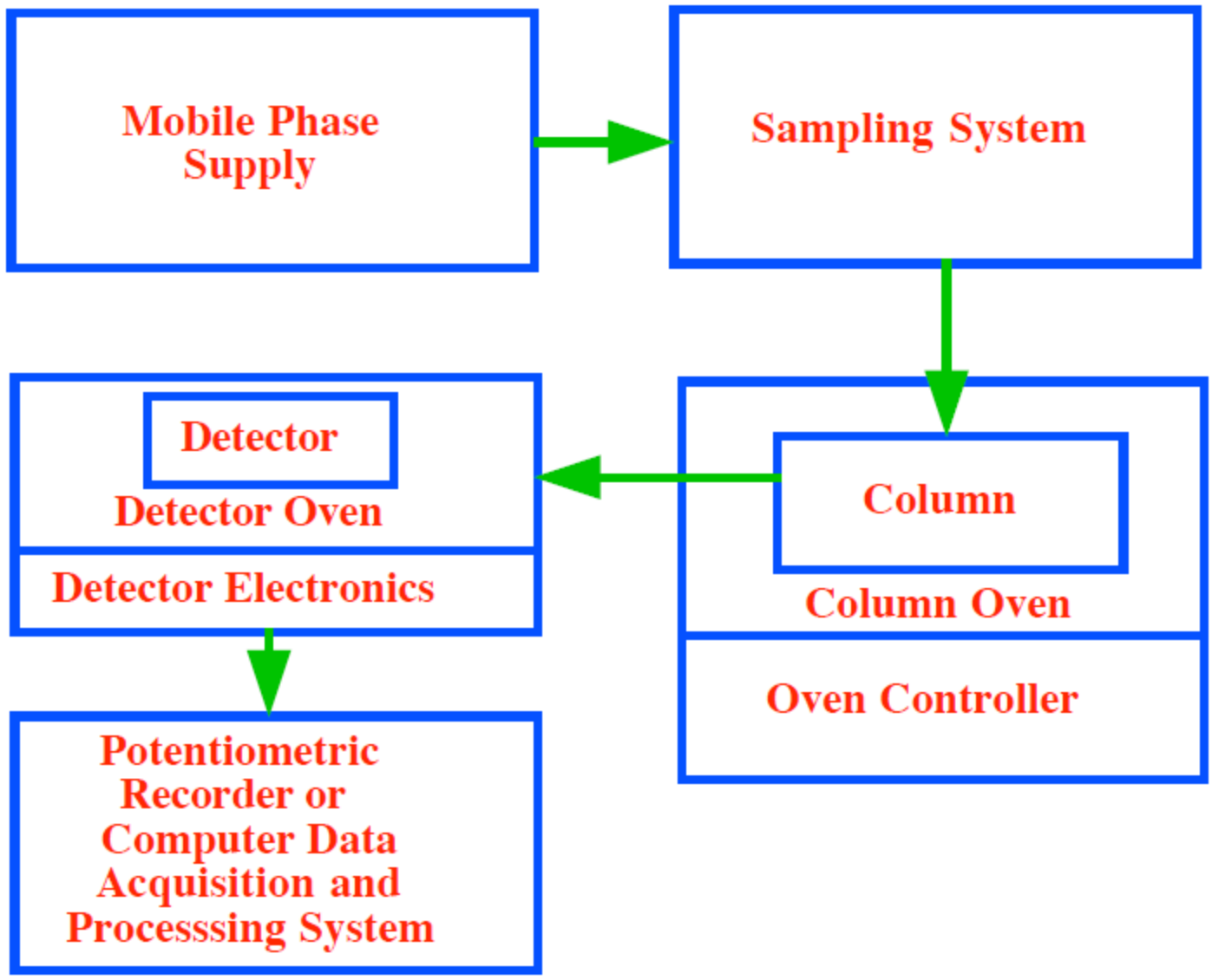
N is number of Theoretical plates. Again the bigger the N the better, because that means the width of the peak is small compared to its retention time.

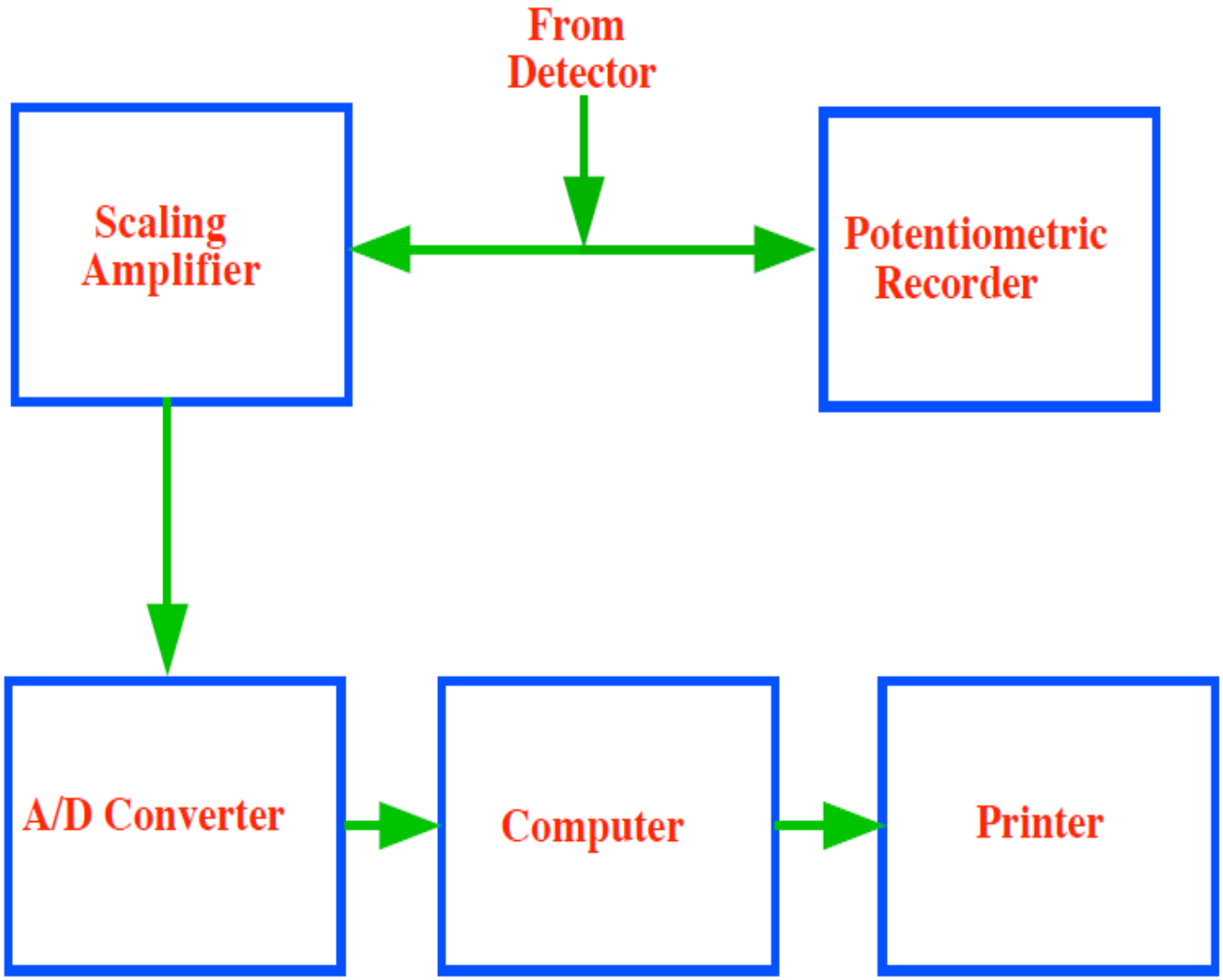
PRICNIPLES OF CHROMATOGRAPGHY

- Physical method of separation that distributes components to separate between two phases moves in a definite direction.

- Substances are separated based on their differential distribution between two phases

- Substances will move with the mobile phase at different rate depending upon their Partition or Distribution co-efficients.


Mobile Phase Supply
Sampling System
Detector
Detector Oven
Detector Electronics
Column
Column Oven
Oven Controller
Potentiometric Recorder or Computer Data Acquisition and Processsing System


From
Detector
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Amplifier
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Recorder
A/D Converter
Computer
Printer

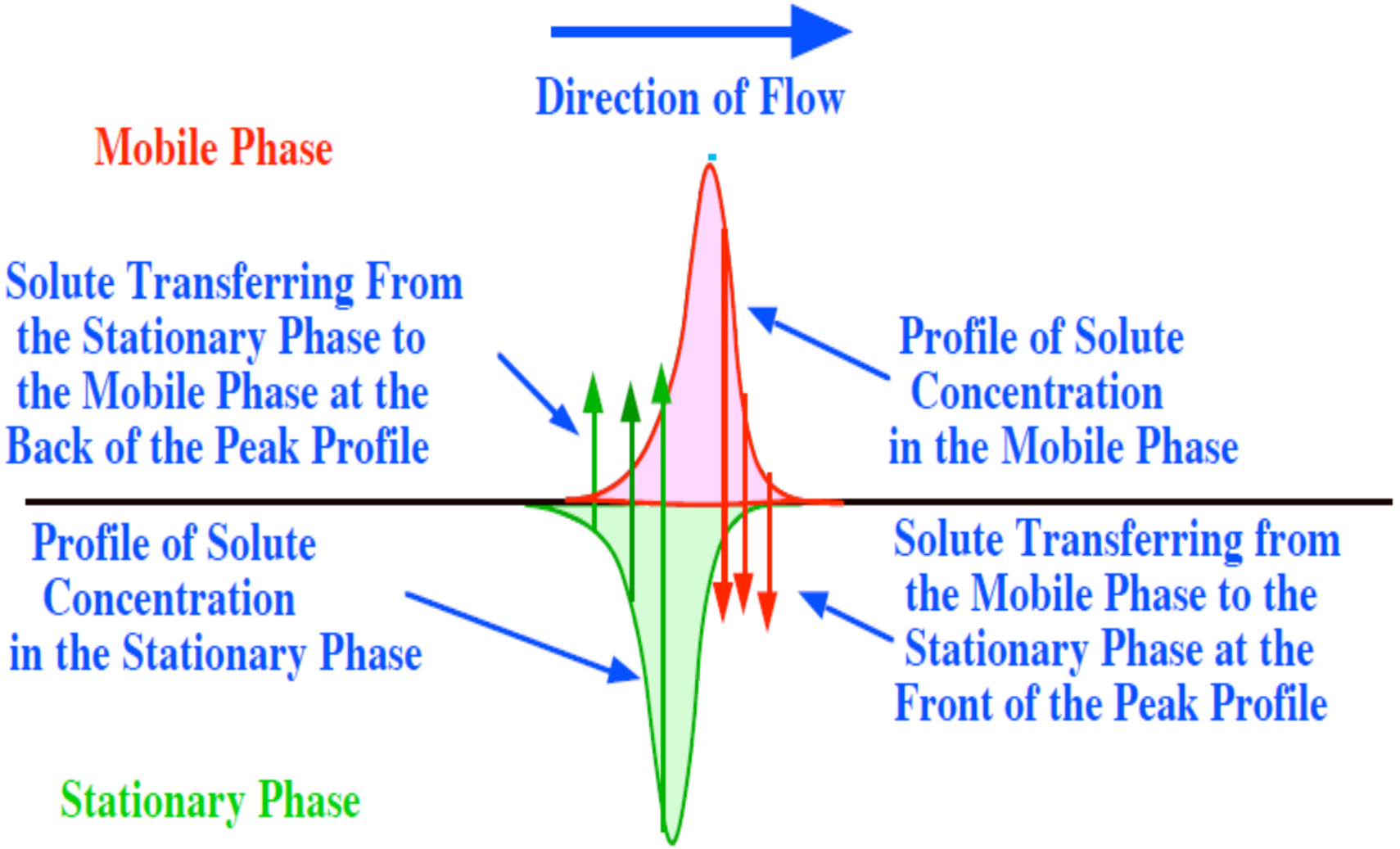


Figure 1. The Elution of a Solute Through a Chromatographic System

FACTORES AFFECTING THE SEPARATION

- Intermolecular interaction between the two phases
- Extent of dispersion of solute molecules over the stationary phase

CLASSIFICATION OF CHROMATOGRAPHY

- Techniques by Chromatographic bed shape
  - Column chromatography
  - Planar chromatography
    - Paper chromatography
    - Thin layer chromatography
- Techniques by Physical state of mobile phase
  - Gas chromatography
  - Liquid chromatography
- Affinity chromatography
  - Supercritical fluid chromatography

TECHNIQUES BY CHROMATOGRAPHIC BED SHAPE

A.COLUMN CHROMATOGRAPHY

PRINCIPLES

- Solid materials (Adsorbants) – Ability to hold the molecules at their surface
- Attractive forces (Vanderwalls & Hydrogen)
- Functional groups (Hydroxyl/ Aromatic)
- Silica

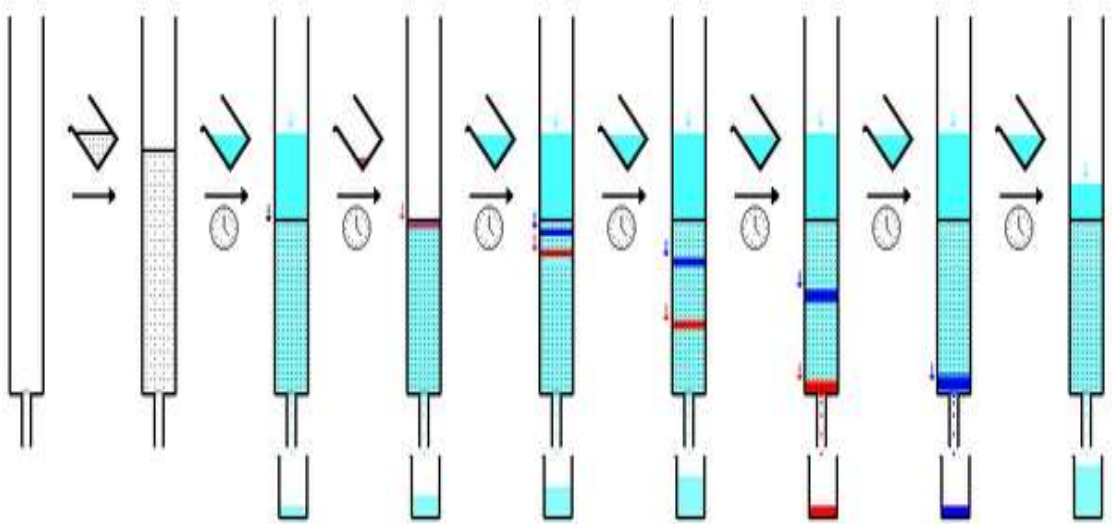
- Stationary bed is within a tube.
- Solvent is driven through the column by applying Positive pressure.
- Separations - 20 minutes

- Modern flash chromatography :

➢ Pre-packed plastic cartridges,
➢ Solvent is pumped through the cartridge.
➢ Quicker separations
➢ Less solvent usage.

- **Column :**
  - Diameter - 5 mm to 50 mm
  - Height - 5 cm to 1 m with a tap
  - Filter (a glass frit or glass wool plug)
- The individual components are retained by the stationary phase differently and separate from each other while they are running at different speeds through the column with the eluent.
- During the entire chromatography process the eluent is collected in a series of fractions. The composition of the eluent flow can be monitored and each fraction is analyzed for dissolved compounds, e.g., UV absorption, or fluorescence.

STATIONARY PHASE



- Silica gel, Alumina. Cellulose

SOLVENTS

- Hydroxyl groups - Alcohol
- Carboxyl group - Acetone
- Non polar Compounds – Hexane
  Heptane
  Toulene

- Flow rate - Separation.
- Pump or compressed gas (e.g. Air, Nitrogen, Argon)
- A faster flow rate of the eluent:
  - Minimizes the time required to run a column
  - Minimizes diffusion
  - Better separation.

Retention Time: The time from the start of signal detection by the detector to the peak height of the elution concentration profile of each different sample.

Curve Width: The width of the concentration profile curve of the different samples in the chromatogram in units of time.

RESOLUTION (R_S) :

$$R_s = 2(t_{RB} - t_{RA})/(w_B + w_A)$$

Where:

t_{RB} = Retention time of solute B

t_{RA} = Retention time of solute A

w_B = Gaussian curve width of solute B

w_A = Gaussian curve width of solute A

Plate Number (N):

$$N = (t_R)^2/(w/4)^2$$

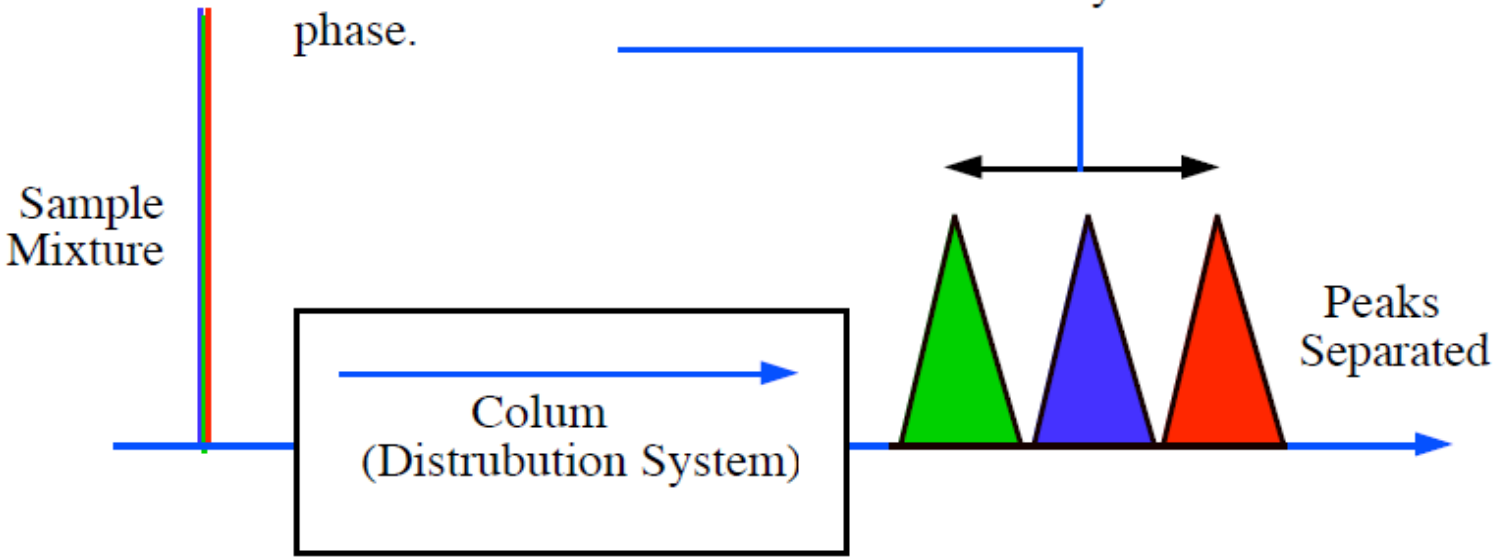
Plate Height (H):

$$H = L/N$$

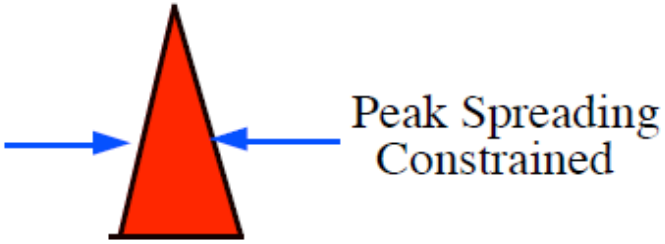
Where L is the length of the column.

Two Processes Occur in the Column

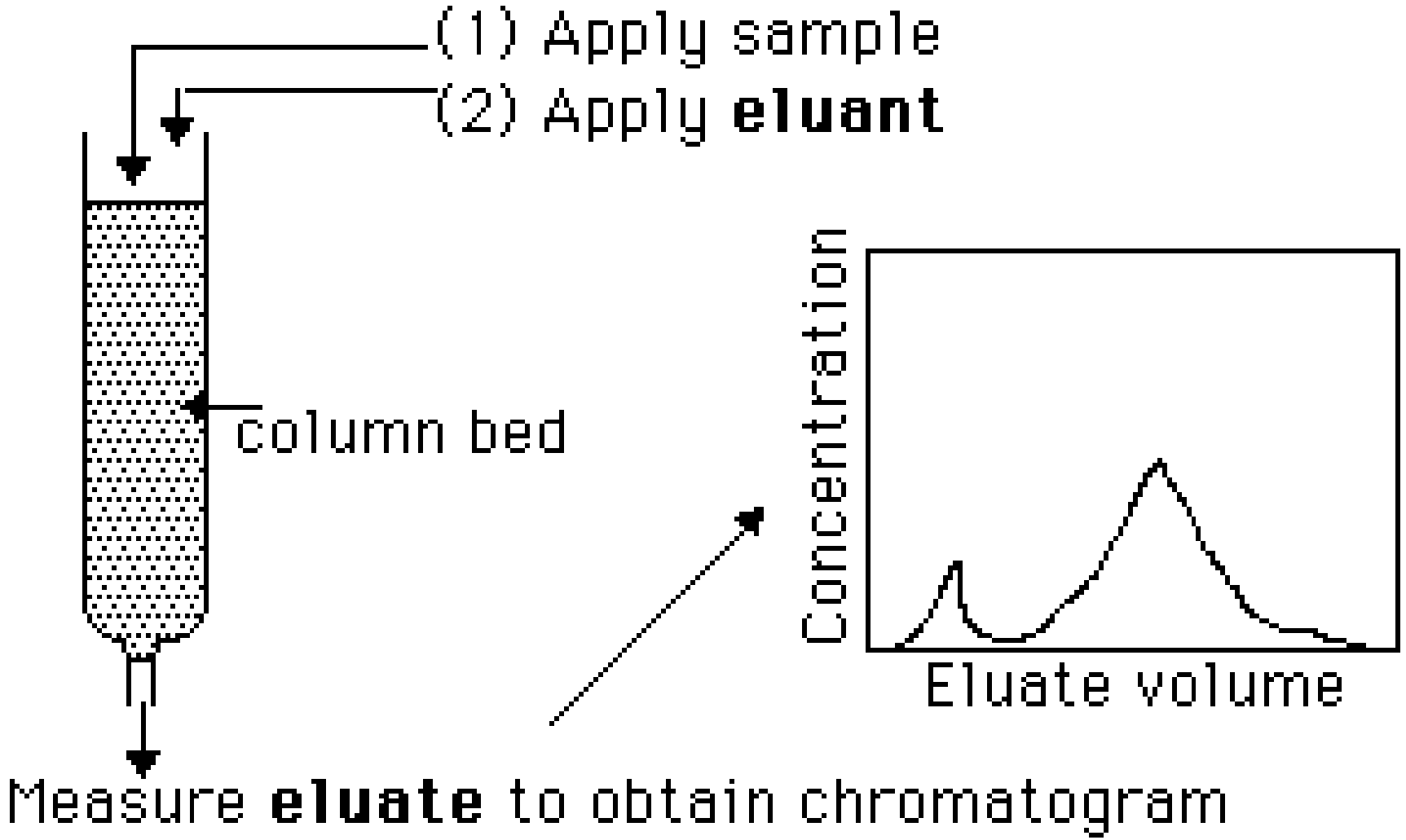
1 The peaks are moved appart as a result of their relative affinities for the stationary phase.



2 The spread (dispersion) of the peaks is constrained so that the solutes can be eluted discretely.

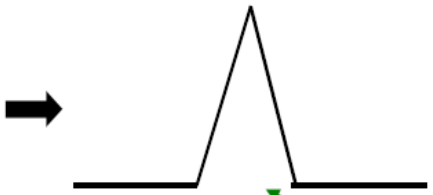


The Function of the Column


(1) Apply sample
(2) Apply **eluant**
column bed
Concentration
Eluate volume
Measure **eluate** to obtain chromatogram

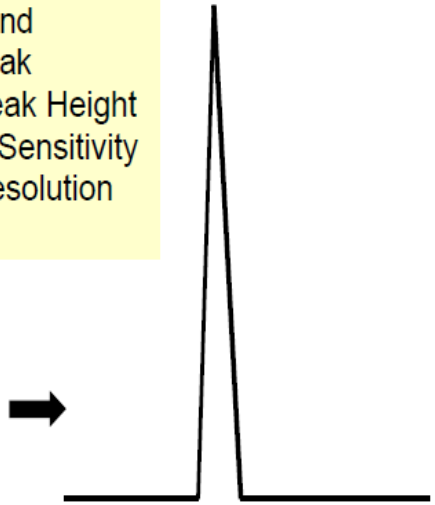
Less Plates

Broad Band – Broad Peak – Less Sensitivity
Less Resolution Capability



More Plates

Narrow Band
Narrow Peak
Greater Peak Height
Increased Sensitivity
More Resolution
Capability

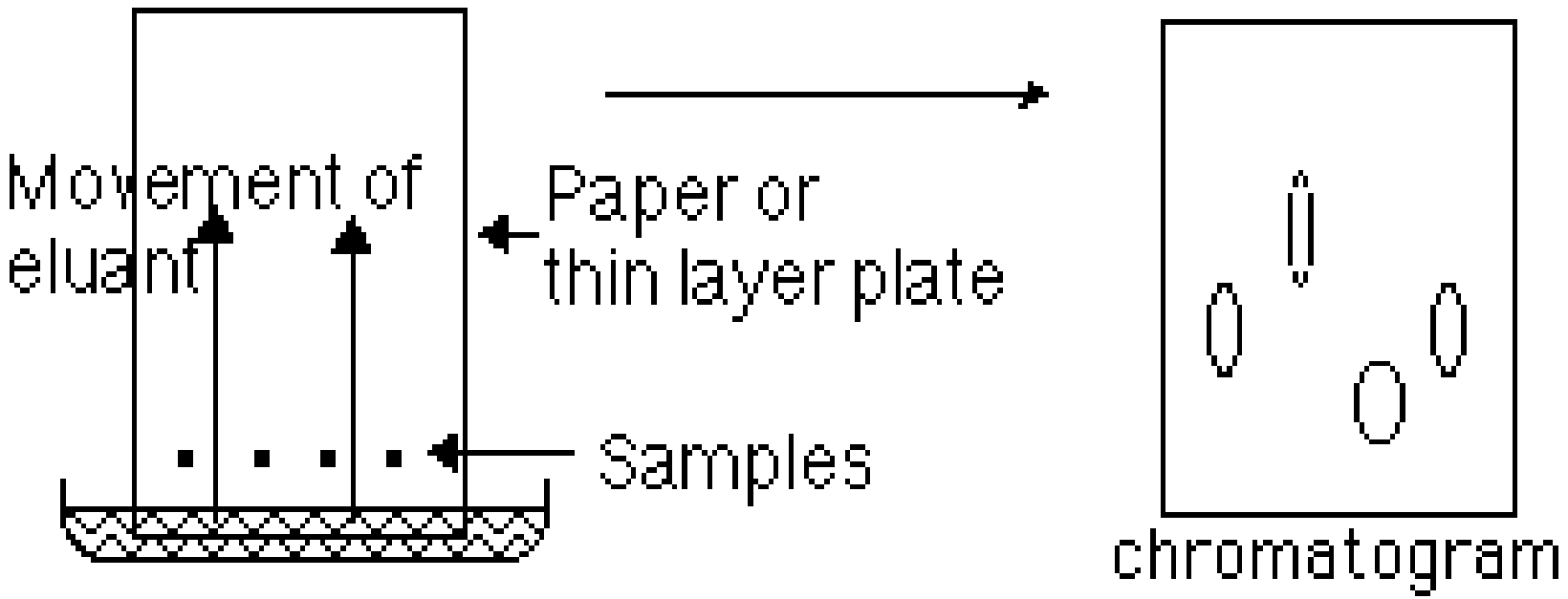


Same Peak Areas

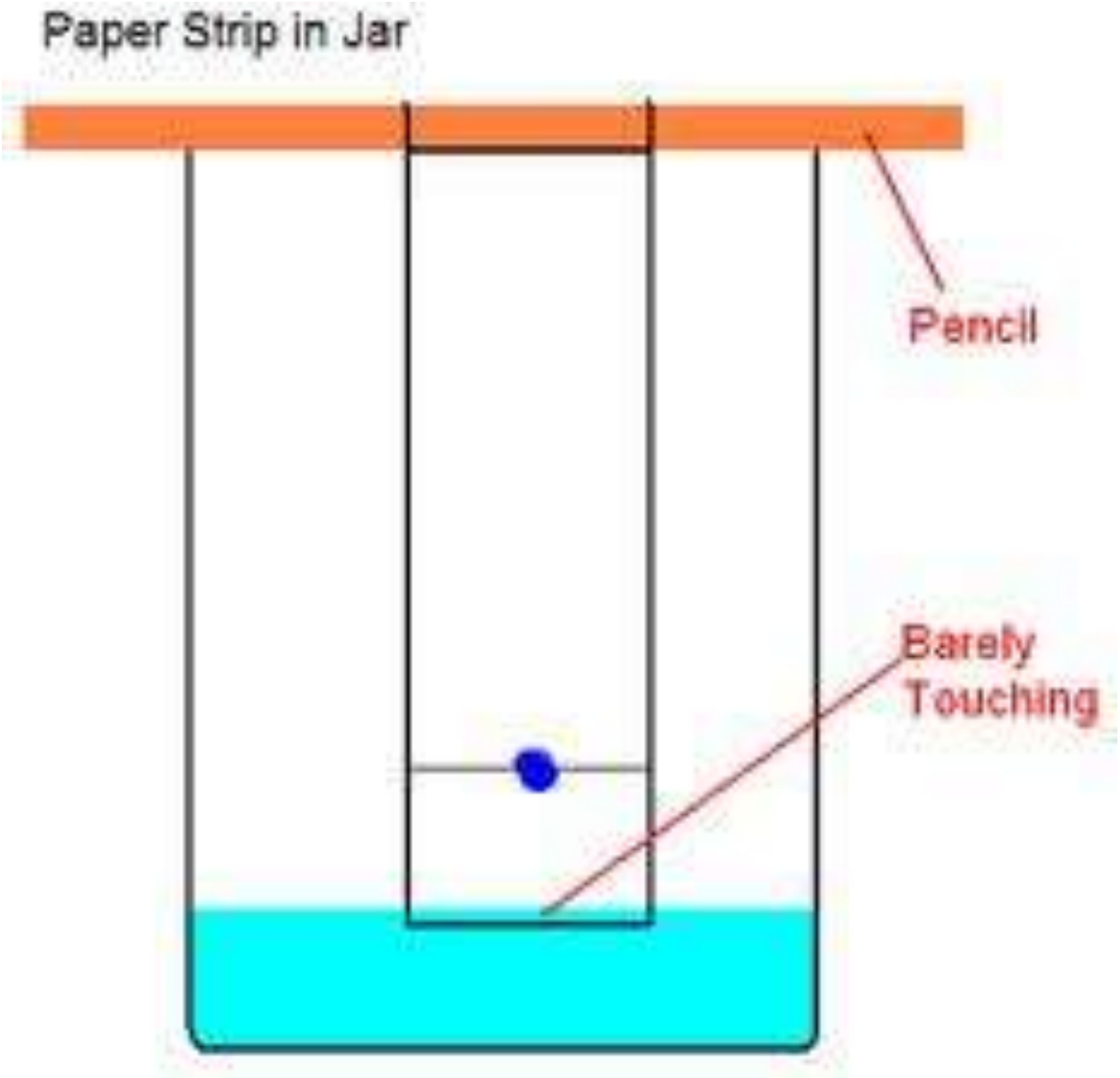
Requires columns with smaller particle sizes and instrumentation to minimize Band Spreading

B. PLANAR CHROMATOGRAPHY

- Separation technique - Stationary phase is present as or on a plane.
- Paper – Paper Chromatography
- Layer of solid particles spread on a support such as a glass plate - Thin layer Chromatography.
- Different compounds in the sample mixture travel different distances according to how strongly they interact with the stationary phase as compared to the mobile phase.
- Retention factor (R_f)


Movement of
eluant
Paper or
thin layer plate
Samples
chromatogram

PAPER CHROMATOGRAPHY

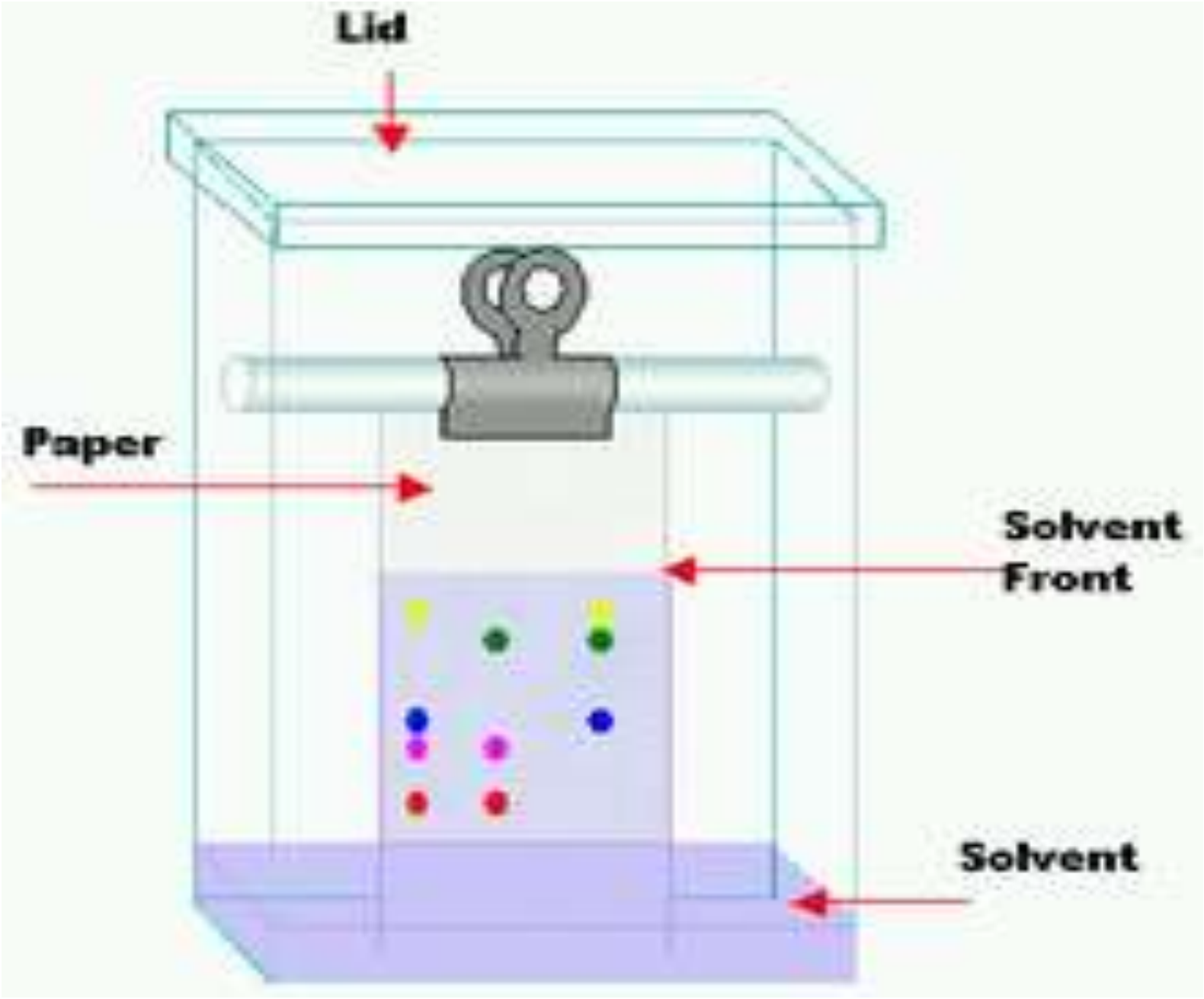

Paper Strip in Jar
Pencil
Barely
Touching

PRINCIPLE

- This paper is made of cellulose, a polar substance, and the compounds within the mixture travel farther if they are non-polar.
- More polar substances bond with the cellulose paper more quickly, and therefore do not travel as far.

"Like Attracts Like – Opposites are Not Attracted"

- Polars attracted to other polars (likes attract)
- Non-polars attracted to other non-polars (likes attract)
- Non-polars have no attraction to polars (opposites repel)


Lid
Paper
Solvent
Front
Solvent

- Retention factor :
- R_f = Distance travelled by a Solute / Distance travelled by a Solvent
- R_f = zero, - Solute remains in the stationary phase and thus it is immobile.
- R_f = 1 - Solute has no affinity for the stationary phase and travels with the solvent front.

- **b) THIN LAYER CHROMATOGRAPHY**

- Widely employed laboratory technique
- Stationary phase - Adsorbent - Silica gel
  Alumina
  Cellulose
- Widely used in pharmaceutical & food stuff industry

❑ Advantages :

✓ Simple, Rapid and Cheap
✓ Faster runs
✓ Better separations
✓ Choice between different adsorbents.
✓ Better resolution
✓ Allow for quantification

- Used to identify the unknown compounds and to determine the purity of mixture.

- TLC Plate - Aluminium or glass - coated by stationary phase.
- Coated material : 0.1-0.3mm in thickness
- Fluorescent indicator that will make it florescence during the UV light exposure.

STATIONARY PHASE

- Silica gel, Alumina, or Cellulose on a flat, inert substrate.

MOBILE PHASE

- Volatile Organic solvents

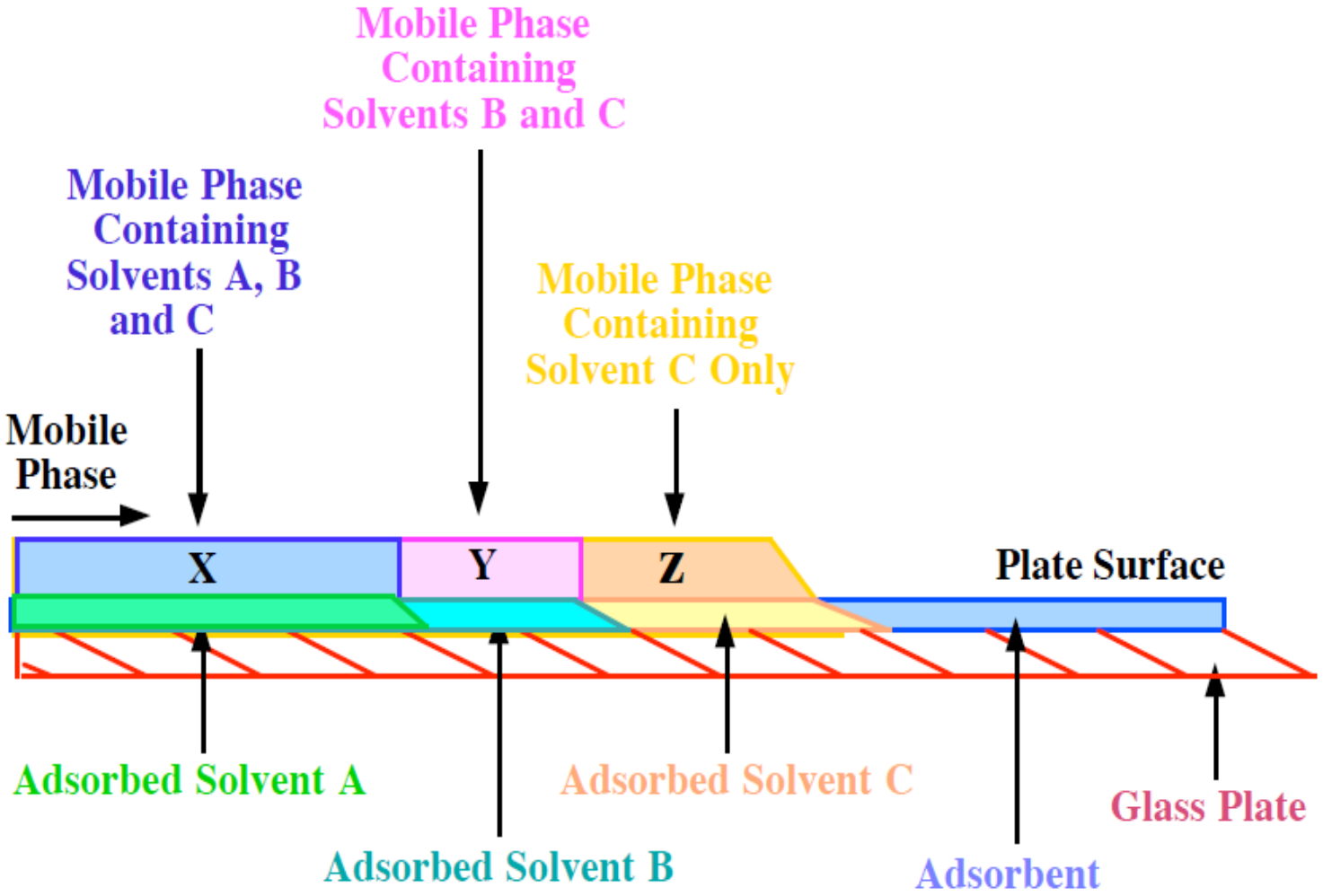
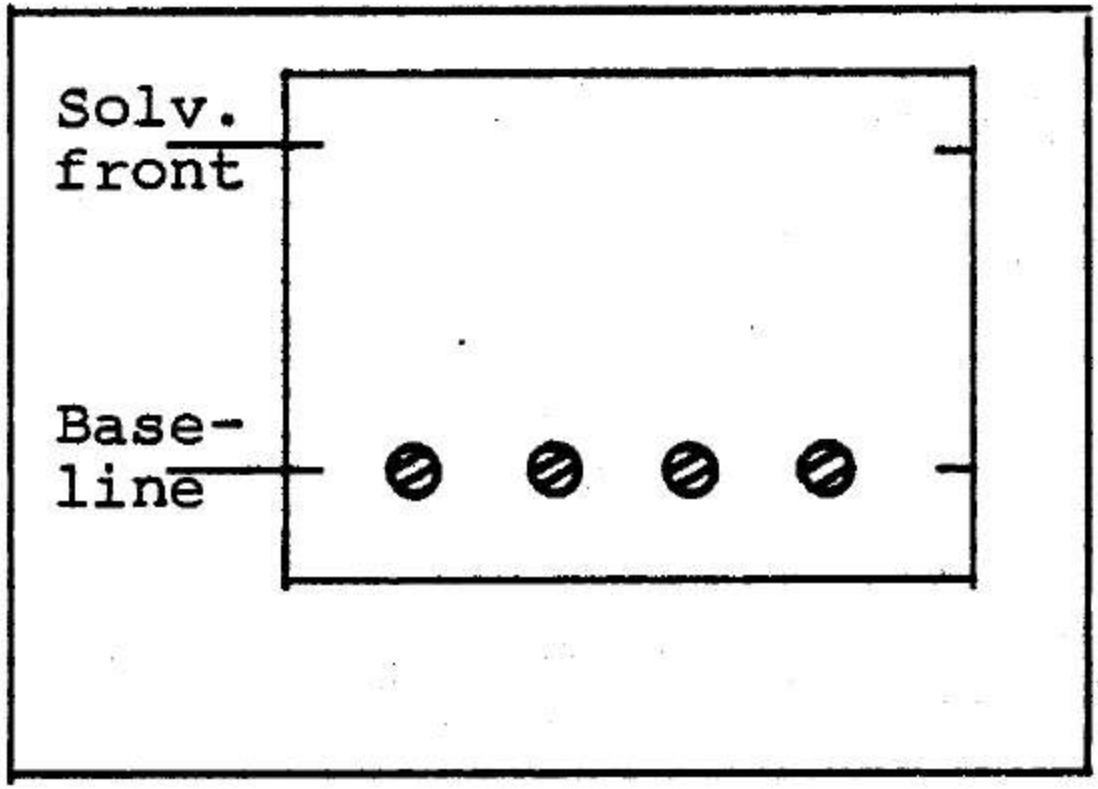


Figure 3. The Development of a Thin Layer Plate

Application of Spots to a TLC plate



SPRAYS

Reagent	Heating treatment	Viewed	Solutes used for
Dragendorff's reagent	-	Daylight	Alkaloids
5% vanillin in H_2SO_4	-	Daylight	Terpenes
60% aqueous sulphuric acid	Heat 120° 10-15 minutes	Daylight and UV	General spray particularly steroids
Antimony trichloride 10% in benzene	Heat 120°C 10 minutes	Daylight and UV	Steroids
Phosphomolybdic acid 10% in 95% ethanol in water	Heat 110° C 5 minutes	Daylight	General spray for unsaturated and oxy-compounds
Aniline hydrogen phthalate	Heat 105°C 10 minutes	Daylight and UV	Sugars
Ninhydrin reagent	Heat 110° until colour develops	Daylight	Amino acids

- **RETENTION FACTOR :**
- R_f = Distance travelled by a Solute
- Distance travelled by a Solvent

- R_f = zero, Solute remains in the stationary phase and
- thus it is immobile.
- R_f = 1 Solute has no affinity for the stationary phase
- and travels with the solvent front.

- **2.TECHNIQUES BY PHYSICAL STATE OF MOBILE PHASE**

A. GAS CHROMATOGRAPHY

- Gas-Liquid chromatography, (GLC)
- Mobile phase – Gas (Helium) Carrier Gas Pressure = 4 kg/cm^2
- Stationary phase - Column, which is typically "packed" or "capillary".
- The stationary phase is adhered to the inside of a small-diameter glass tube (a capillary column) or a solid matrix inside a larger metal tube (a packed column).
- Partition Coefficient of Volatile analyte between a solid stationary phase (Silicone) and a mobile gas (Helium).

- Advantages
- High sensitivity,
- High Resolution,
- High speed
- High Accurasy,
- Highly Quantitative

❑

❑ **APPARATUS**

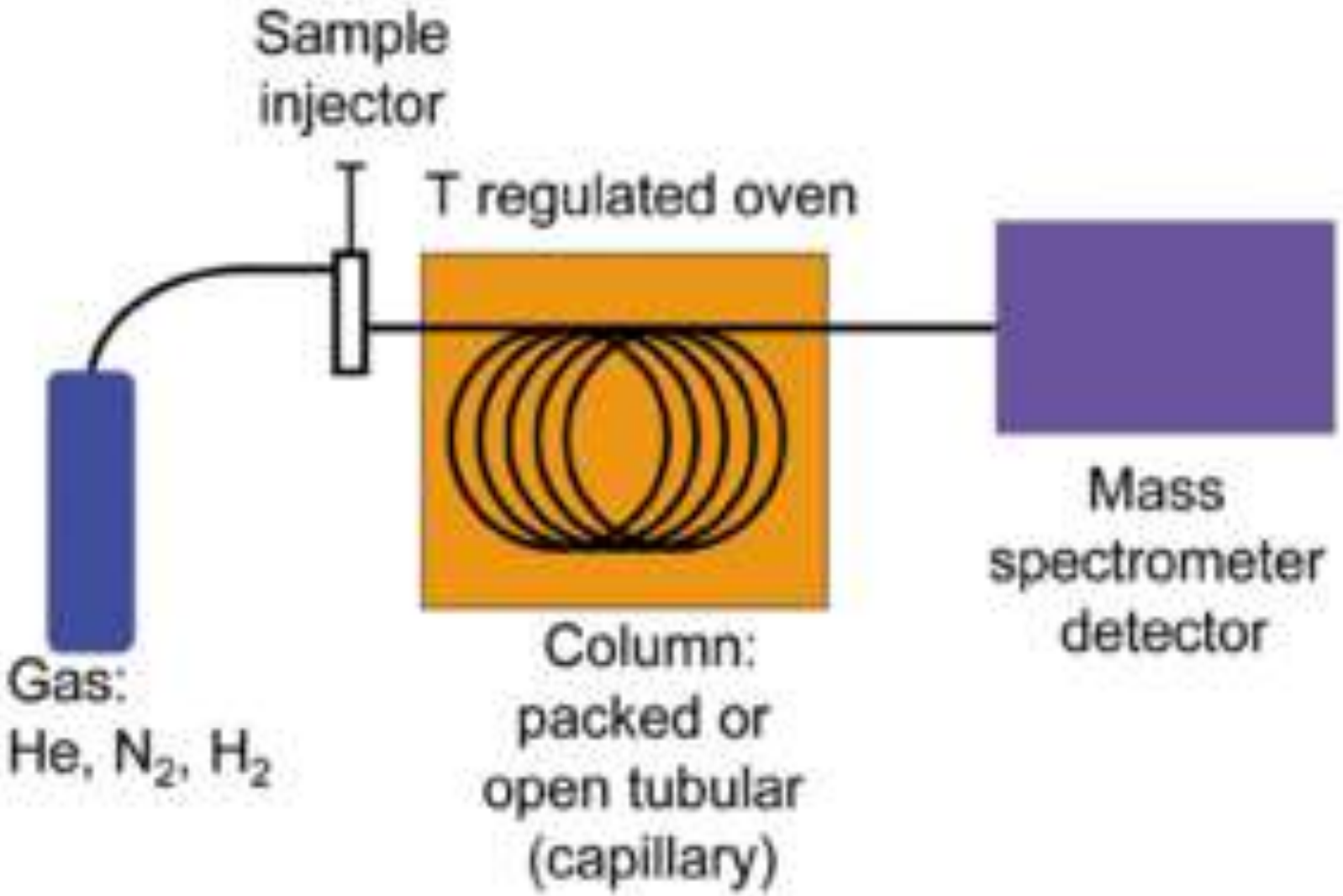
- Gas Chromatograph, GC analyzer, Normal syringes and one micro syringe, Beakers, Sample bottles and Electronic weight.

❑ **CHEMICALS**

- Methanol, Isopropyl Alcohol and water

❑ **SAMPLE:**

- Gases, Liquid, Solids
- M.Wt: 2-800
- Volatile


Sample injector
T regulated oven
Mass spectrometer detector
Gas: He, N_2, H_2
Column: packed or open tubular (capillary)

APPLICATION

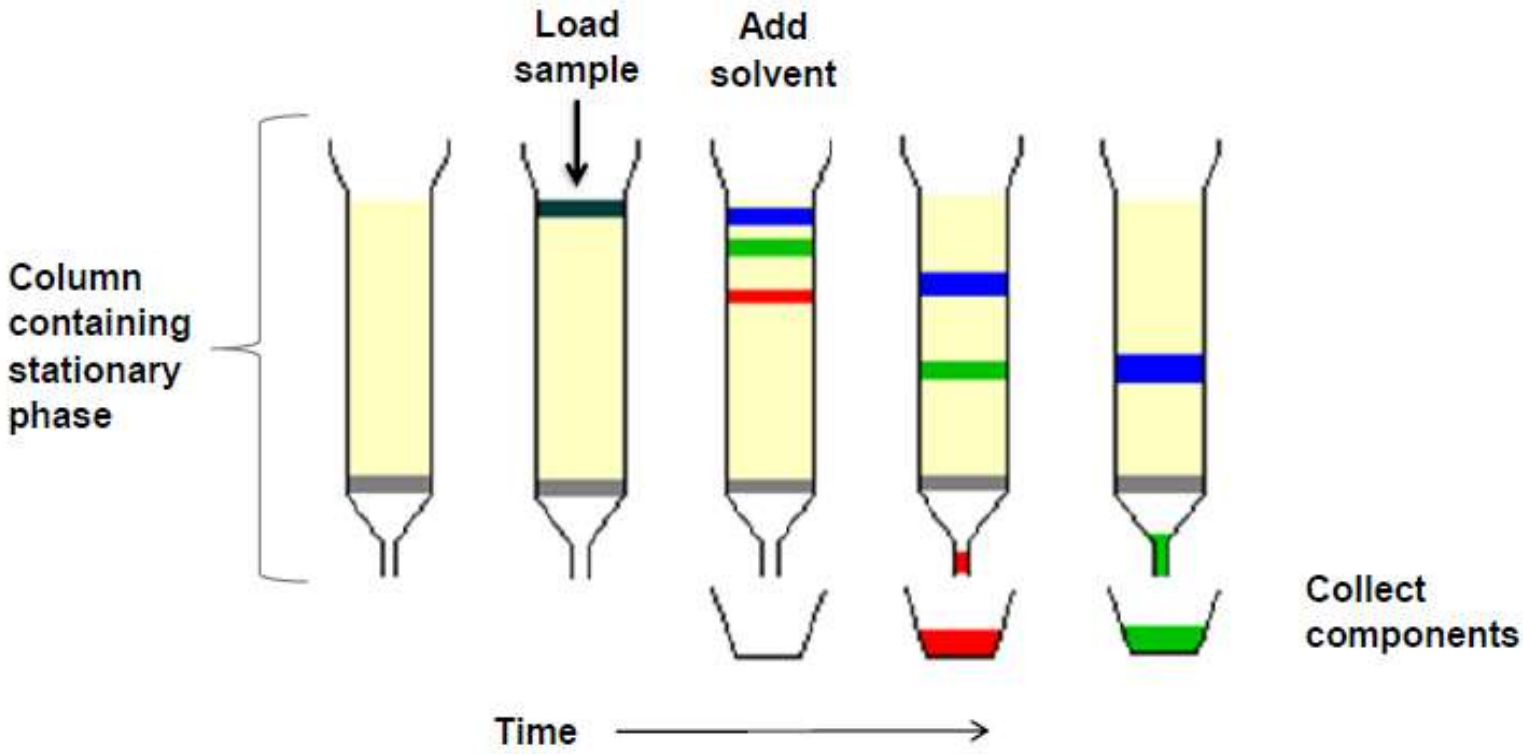
- Quantitative & Qualitative analysis of low polarity compounds
- Analytical chemistry, Biochemistry, Petrochemical, Environmental monitoring
- Measure picomoles of a substance in a 1 ml liquid sample, or parts-per-billion concentrations in gaseous samples
- Measuring toxic substances in soil, air or water.

APPLICATION OF GC- MS

- **Environmental monitoring :** Oraganic Pollutants
- **Criminal forensics :** Analyze the particles (Fibre) from a human body in order to help link a criminal to a crime.
- **Law enforcement :** Detection of illegal narcotics,
- **Forensic toxicology :** Find drugs and/or poisons in biological specimens of suspects, victims, or the deceased.
- **Sports anti-doping analysis :** Test athletes' urine samples
- **Security :** Explosive detection (September 11 development) systems have become a part of all US airports.
- **Food, beverage and perfume :** from spoilage or Adultration - aromatic compounds, esters, fatty acids, alcohols, aldehydes, terpenes
- **Medicine :** Congenital metabolic diseases In Born error of metabolism

- **B. LIQUID CHROMATOGRAPHY**
- Mobile phase - Liquid.
- Column or a plane.
- Very small packing particles and a relatively high pressure - High Performance Liquid Chromatography (HPLC).

Principles of Liquid Chromatography



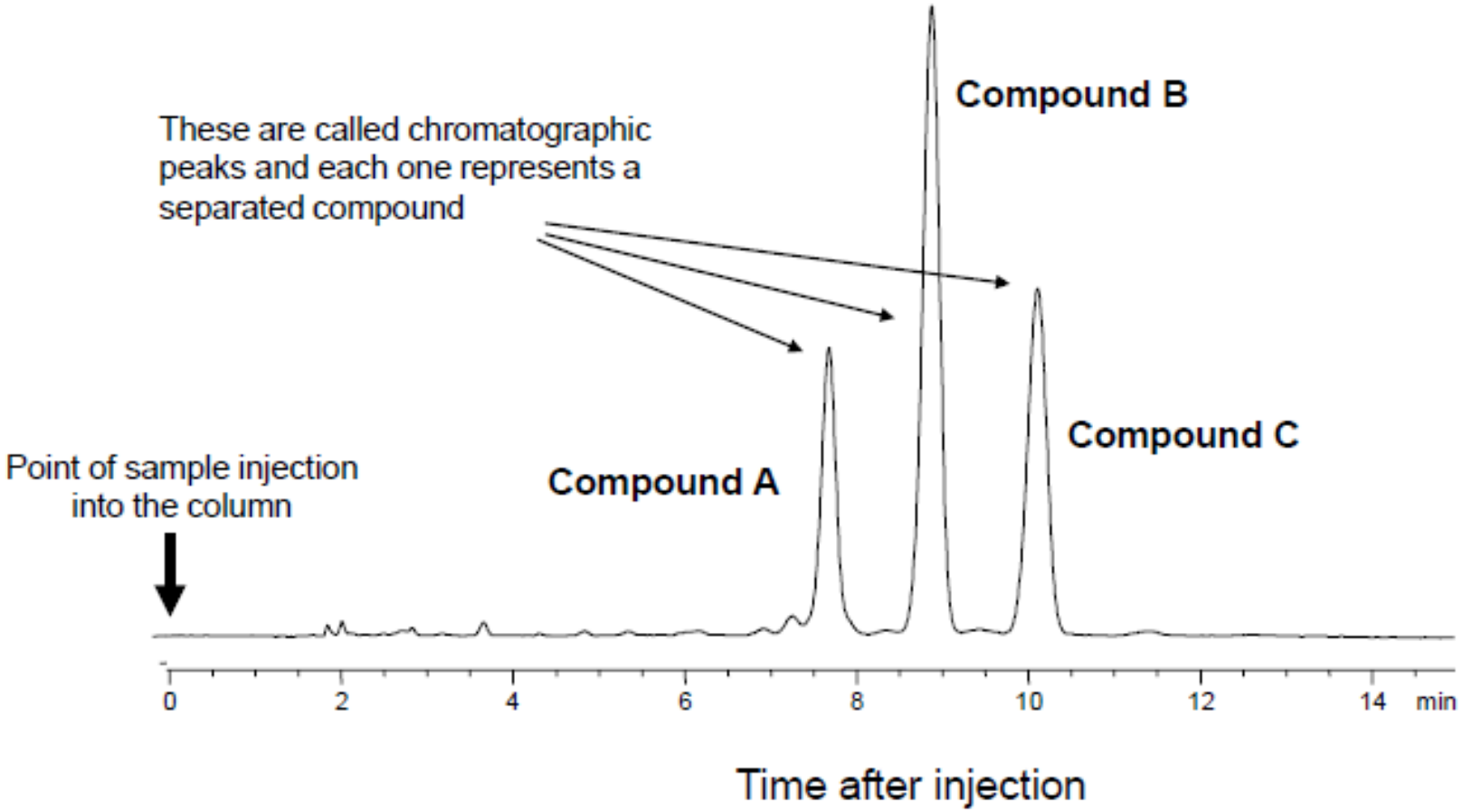
SOLVENT TYPES USED IN L.C.

- ORGANIC
  - Methanol
  - Acetonitrile
  - THF
  - Methylene Chloride
  - Hexane
- AQUEOUS
  - Water
- SOLVENT MIXTURES
  - CHCl3 / ETOH
  - MEOH / H20
  - MEOH / ACN / H20
  - HEXANE / IPA

SOLVENT	UV CUT OFF
n-PENTANE	190
ISO-OCTANE	215
TOLUENE	285
BENZENE	280
CHLOROFORM	245
METHANOL	205
TETRAHYDROFURAN	230
ACETONE	330
ACETONITRILE	190
ISOPROPANOL	205
ETHANOL	210

- **Buffers**
  - **Phosphates**
  - **TRIS**
  - **Acetates**

What Does a Liquid Chromatogram Look Like?



This is the chromatogram resulting from the injection of a small volume of liquid extracted from a vitamin E capsule that was dissolved in an organic solvent. Modern HPLC separations usually require 10- to 30-minutes each.

LC- MS

- Mass spectra is obtained rapidly
- Small amount of material is required to form the spectra.
- Data collected is highly informative with respect to molecular structure.

APPLICATION

- **Pharmacokinetics :** How quickly a drug will be cleared from the hepatic blood flow and organs of the body.
- **Proteomics** **:** Peptide mass fingerprinting
- **Drug development:** Peptide Mapping, Glycoprotein Mapping, Natural Products Dereplication, Bioaffinity Screening, In Vivo Drug Screening, Metabolic Stability Screening, Metabolite Identification, Impurity Identification, Degradant Identification, Quantitative Bioanalysis, and Quality Control.
- **Fungal toxins**
- **Pesticides, Herbicides**

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

HPLC V/S LC TECHNIQUE

- Columns : Small diameter (4.6 mm), stainless steel, glass or titanium.
- Column packing with very small (3, 5 and 10 μm) particles
- Relatively high inlet pressures and controlled flow of the mobile phase.
- Detecting very small amounts
- High resolution
- Rapid analysis
- Speed, efficiency, sensitivity and ease of operation
- High degree of versatility
- Easily separate a wide variety of chemical mixtures

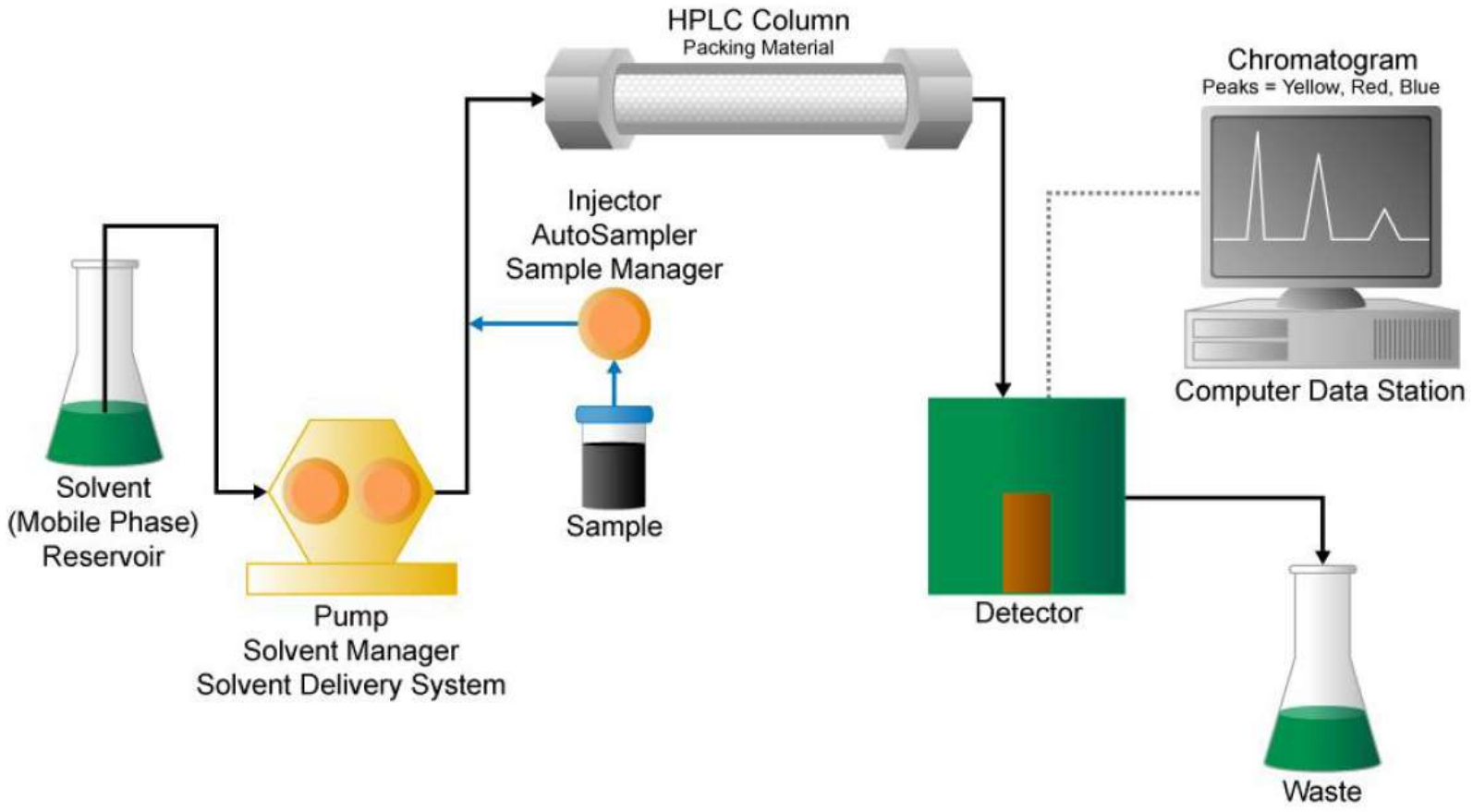
- 400 atmospheres.

PUMP PRESSURE

- "Ultra High Performance Liquid Chromatography" systems 1000 atmospheres.

HPLC System

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™



- **ELUTION :** Isocratic and Gradient.

❑ **ISOCRATIC :**

- ISO ==> SAME
- - Solvent Composition Stays the *Same for the Entire Run*

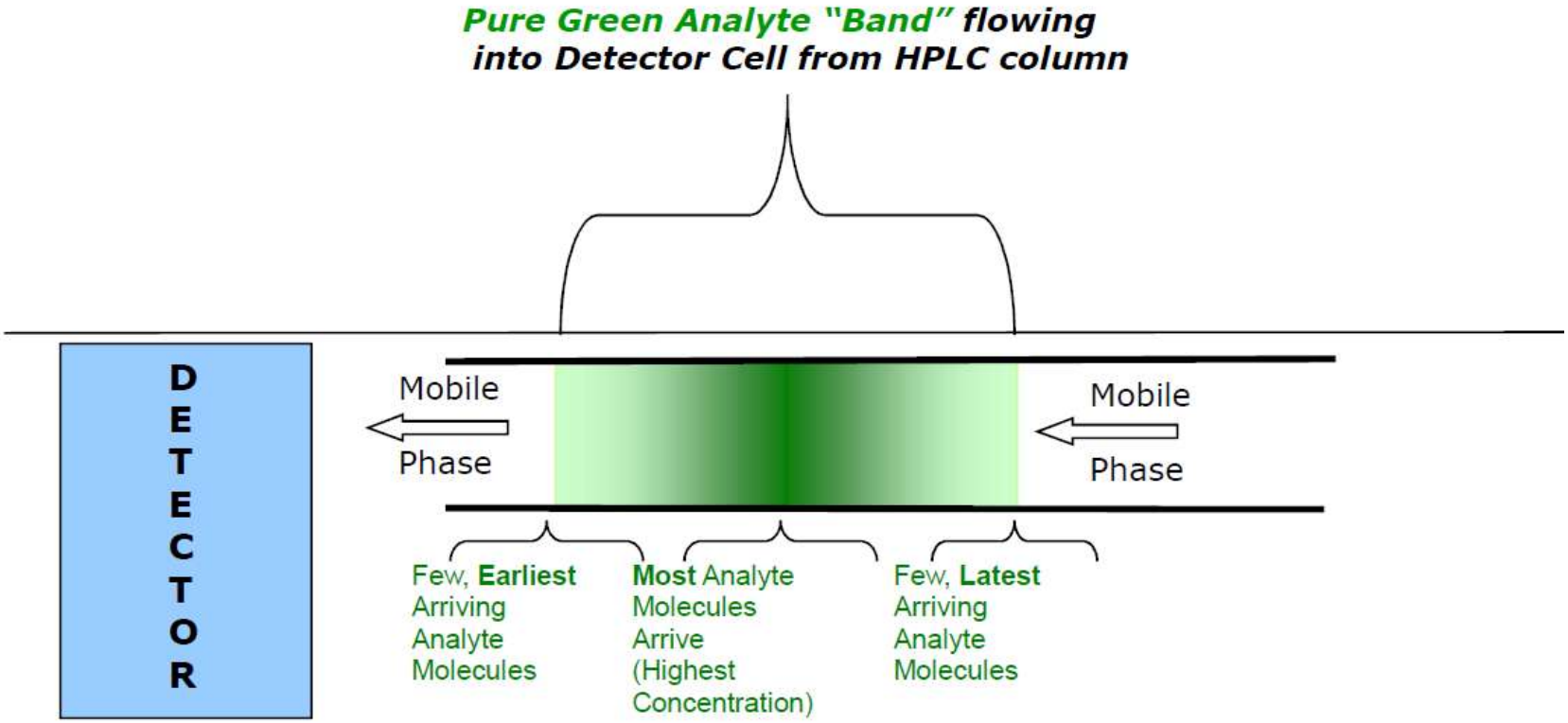
  EX: 60:40 Alcohol:Water

❑ **GRADIENT :**

- Solvent Composition *Changes Throughout the Run*

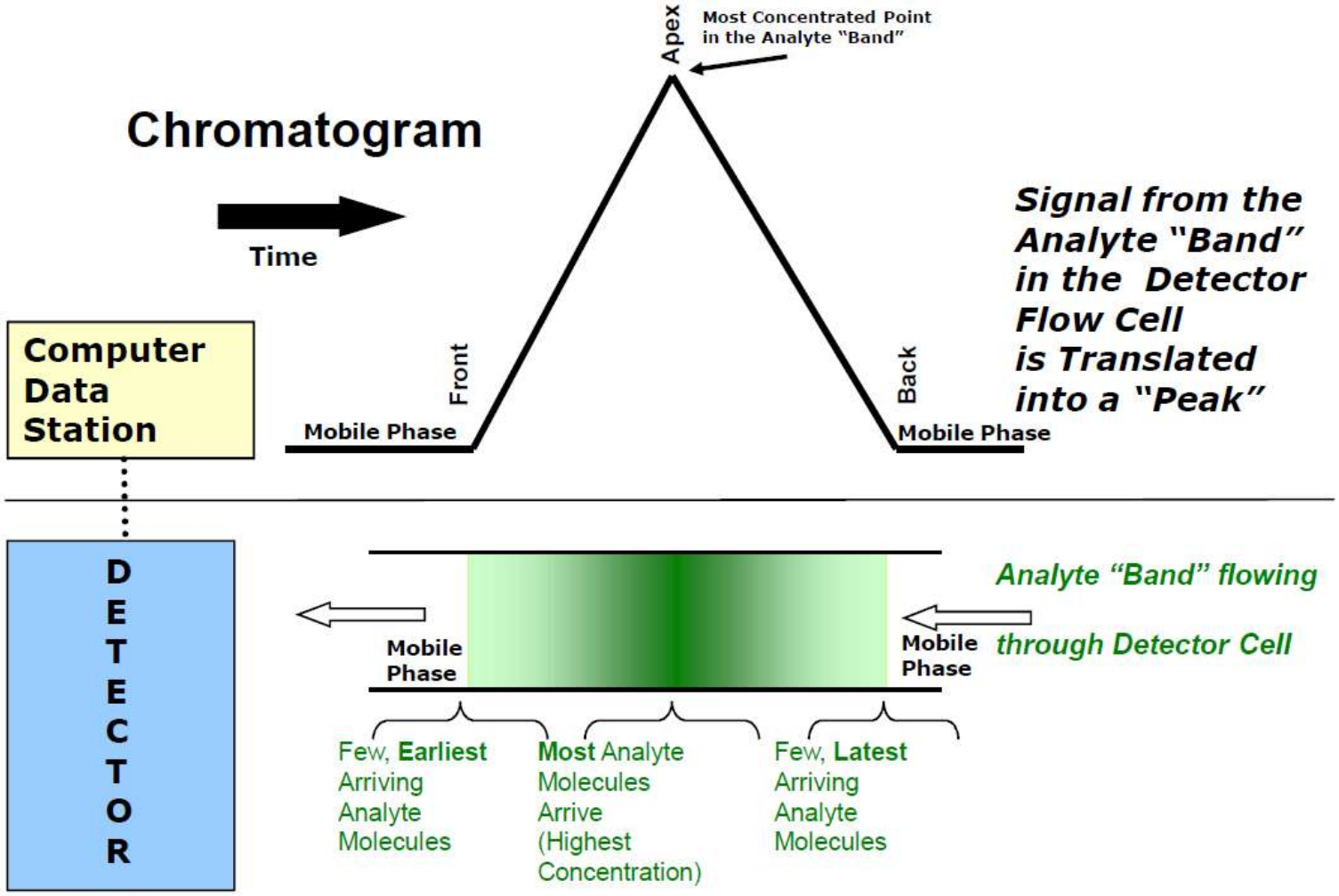
How an Analyte Band becomes a Peak

Waters
THE SCIENCE OF WHAT'S POSSIBL



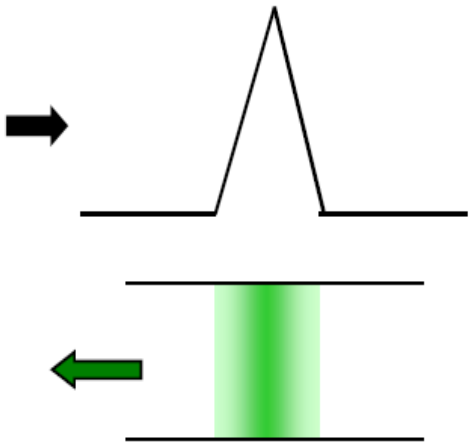
How an Analyte Band becomes a Peak

Waters
THE SCIENCE OF WHAT'S POSSIBL



HPLC

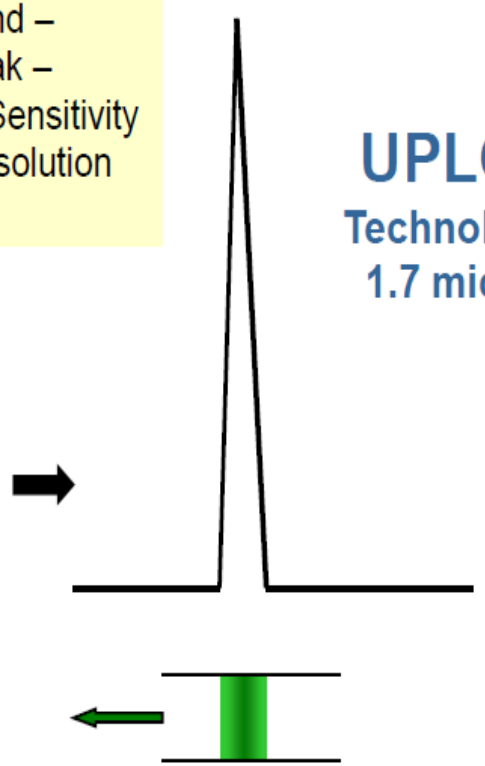
Broad Band – Broad Peak – Less Sensitivity



The Population of Green Analyte molecules arrive at much different times

Narrow Band –
Narrow Peak –
Increased Sensitivity
More Resolution
Capability

UPLC®
Technology
1.7 micron



The Population of Green Analyte molecules all arrive **closer** in time, more concentrated

Requires columns and instrumentation to minimize Band Spreading

TYPES OF HPLC

- ✓ Nature of the stationary phase
- ✓ Separation process

- ❑ Adsorption chromatography
- ❑ Ion-exchange chromatography
- ❑ Size exclusion chromatography

APPLICATION

- Protein separation
  - Insulin purification
  - Plasma fractionation
  - Enzyme purification

SIZE EXCLUSION CHROMATOGRAPHY

- **Gel filtration or gel permeation chromatography**
- Separation - Molecular size of its components.
- Larger molecules are rapidly washed through the column, smaller molecules penetrate inside the porous of the packing particles and elute later.

APPLICATIONS

Field	Typical Mixtures
Pharmaceuticals	Antibiotics, sedatives, steroids, analgesics
Biochemical	Amino acids, proteins, carbohydrates, lipids
Food products	Artificial sweeteners, antioxidants, aflatoxins, additives
Industrial chemicals	Condensed aromatics, surfactants, propellants, dyes
Pollutants	Pesticides, herbicides, phenols, polychlorinated biphenyls
Forensic science	Drugs, poisons, blood alcohol, narcotics
Clinical chemistry	Bile acids, drug metabolites, urine extracts, estrogens

- *Pharmaceuticals*
- *Environmental*
  - *Contamination*
    - *Herbicides (Weed Killers – Round-up®)*
    - *Pesticides (Insecticides – Diazanon®)*
    - *Industrial Waste Industrial Products*
- *Industrial Products*
- *Toxins from Molds/Fungus*
- *Antibiotics Overuse (Risk for resistant bacteria strains)*
- *Processing of Food and Food Ingredients/Beverages*
- *Forensic Science*

- **AFFINITY CHROMATOGRAPHY**

- Based on specific & non-covalent binding of the proteins to other molecules – Ligands (His-tags, biotin or antigens)
- Physical properties of the analyte.

- Biochemistry in the purification of proteins **(Enzymes)** bound to tags.

- After purification, some of these tags are usually removed and the pure protein is obtained.

- **SUPERCRITICAL FLUID CHROMATOGRAPHY**
- Used for the analysis and purification of low to moderate molecular weight , thermally labile molecules.
- Principles are similar to those of (HPLC)
- Mobile phase - High pressure liquid or Super critical Carbon Dioxide.
- Modifiers – Methanol, Ehanol, isopropyl alcohol, acetonitrile and Chloroform.

❑ **APPLICATION**

❑ Use in industry primarily for separation of Chiral (Asymmetric Carbon atoms) molecules.

- Serine
- Soman
- Glyceraldehyde
- Phosphours (Phosphine)
- Sulfar metal
- Cobalt
- Enkephalins

DETECTOR

- Gas Chromatography or liquid Chromatography
- To visualize components of the mixture being eluted off the chromatography column.

DETECTORS

- Flame ionization detector
- Aerosol-based detector
- Flame photometric detector (FPD).
- Atomic-emission detector (AED).
- Mass spectrometer (MS) detector
- Nitrogen Phosphorus Detector,
- Evaporative Light Scattering Detector (ELD) : LC.

DETECTORS

- UV detectors
- Thermal conductivity Detector, (TCD)
- Fluorescence detector
- Electron Capture Detector, (ECD)
- Photoionization Detector, (PID)
- Refractive index Detector (RI or RID)
- Radio flow Detector
- Chiral Detector

Uses of Chromatography

Type of Chromatography	Applications in the Real World	Why and What is it
Liquid Chromatography	test water samples to look for pollution,	Used to analyze metal ions and organic compounds in solutions. It uses liquids which may incorporate hydrophilic, insoluble molecules.
Gas Chromatography	detect bombs in airports, identify and quantify such drugs as alcohol, used in forensics to compare fibres found on a victim	Used to analyze volatile gases. Helium is used to move the gaseous mixture through a column of absorbent material.
Thin-Layer Chromatography	detecting pesticide or insecticide residues in food, also used in forensics to analyze the dye composition of fibers	Uses an absorbent material on flat glass plates. This is a simple and rapid method to check the purity of the organic compound
Paper Chromatography	separating amino acids and anions, RNA fingerprinting, separating and testing histamines, antibiotics	The most common type of chromatography. The paper is the stationary phase. This uses capillary action to pull the solutes up through the paper and separate the solutes.

ATOMIC ABSORPTION SPECTROSCOPY

INTRODUCTON:

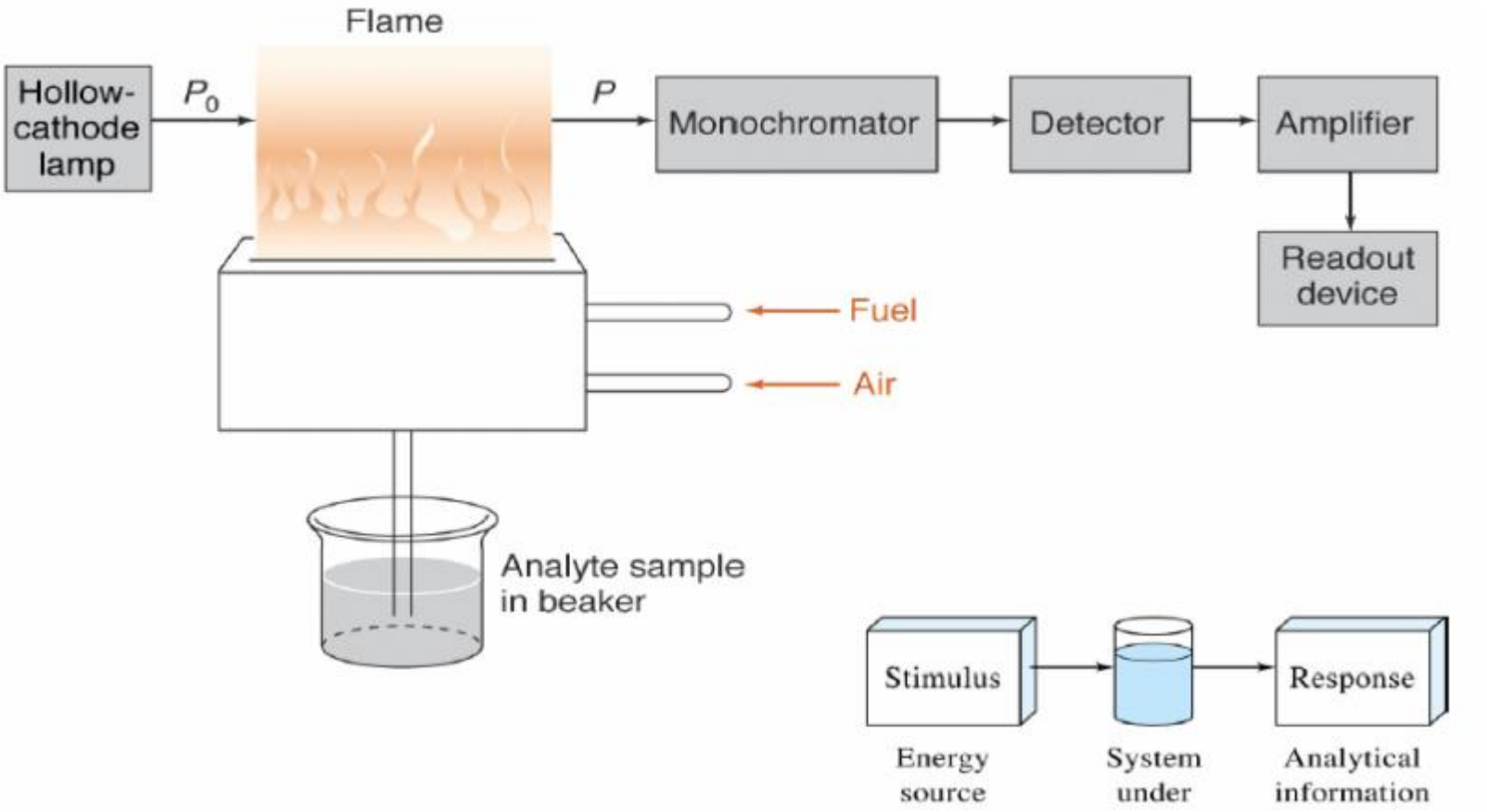
- Atomic absorption spectroscopy is deals with the absorption of specific wave length of of radiation by neutral atoms in the ground state. This phenomenon is similar to UV spectroscopy, where absorption of radiation by molecules occur.
- Neutral atoms are obtained by spraying the sample solution of element using a burner. Specific wavelength of radiation is generated by using a hollow cathode lamp. for determination of every element , separate hollow cathode lamp is required.

PRINCIPLE:

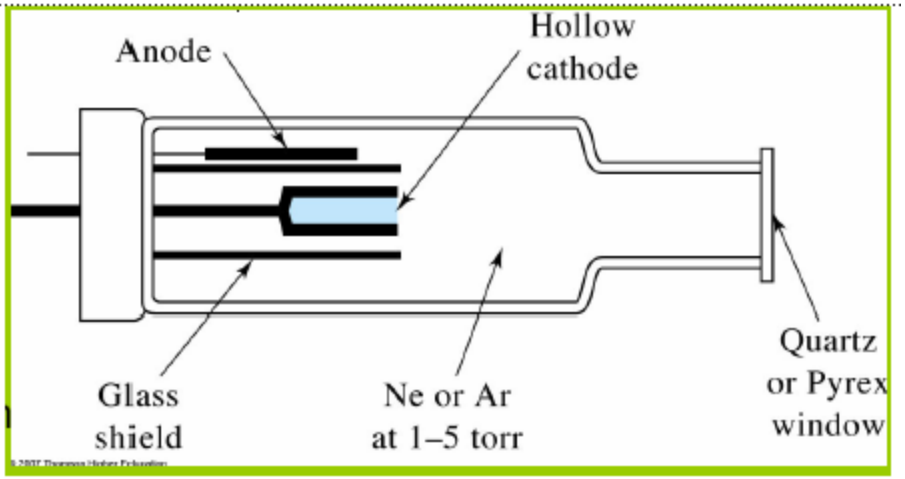
- When solution of metalic salt is sprayed on to a flame, fine droplets are formed , due to the thermal energy of the flame , the solvent in the flame is evaporated , leaving a fine residue, which are converted to neutral atoms.
- These neutral atoms absorb radiation of specific wavelength , emitted by hollow cathode lamp(HCL).hollow cathode lamp is filled with the vapour of element , which gives specific wavelength of radiation.
- For the determination of every element, hollow cathode lamp is selected, which contains vapour of the element to be analysed although this appear to be demerits of AAS , specificities can be achieved only by the use of HCL.

- The intensity of light absorbed by the neutral atom is directly proportion to the concentration of the element and obeys Beer's law over a wide concentration range.
- The intensity of radiation absorbed by neutral atoms is measured using photometric detectors (PMT)
- In AAS the temperature of the flame is not critical , since the thermal energy of flame isused to atomise the sample solution to fine droplets , to form a fine residue and later to neutral atoms.
- The exitation of neutral atoms is brought about only by radiation from hollow cathode lamp and not by the thermal energy of the flame.

INSTRUMENTATION



HOLLOW CATHODE LAMP



•The lamp or source of light in AAS is a hollow cathode lamp.

• The cathode is made up of specific element or alloys of elements or coating of element on cathode.

• When current of 500 V is applied between anode and cathode, metal atoms emerge from hollow cup and collides with filler gas which is argon or neon

• Due to these collisions, numbers of metal atoms are exited and emitt their characteristic radiation .

- These characteristic radiation is absorbed by neutral atoms of the same element in ground state , which occur in the flame, when sample solution is sprayed.
- It is not possible to use a source of light with a monochromator because this arrangement gives a radiation with a band width of 1nm, where as the hollow cathode lamp gives a band width of 0.001 to 0.01nm, which is highly desirable to achieve specificity.
- Moreover, light source should provide a line width less than the absorption line width of the element to be determined

BURNER (WITH FUEL AND OXIDANT):

- There are different burners are available, which are used to spray the sample solution into fine droplets, mix with fuel and oxidant , so that a homogeneous flame of stable intensity is obtained.
- The most common burners are
    1. TOTAL CONSUMPTION BURNER
    2. LAMINAR FLOW BURNER
- If the temperature of the flame is too low, it may not cause exitation of neutral atoms. If temperature is too high , it may cause ionisation of atoms and thus sufficient atoms in exited state may not occur.
- This makes it necessary to select ideal combination of oxidant and fuel which gives the desired temperature.

CHOPPER:

- The chopper in the instrument is rotate like a fan , allows alternatively radiation from flame alone or the radiation from HCL and the flame.
- This produces a pulsating current or signal, which is used to measure the intensity of light absorbed by elements, without interference by radiation from the flame itself.

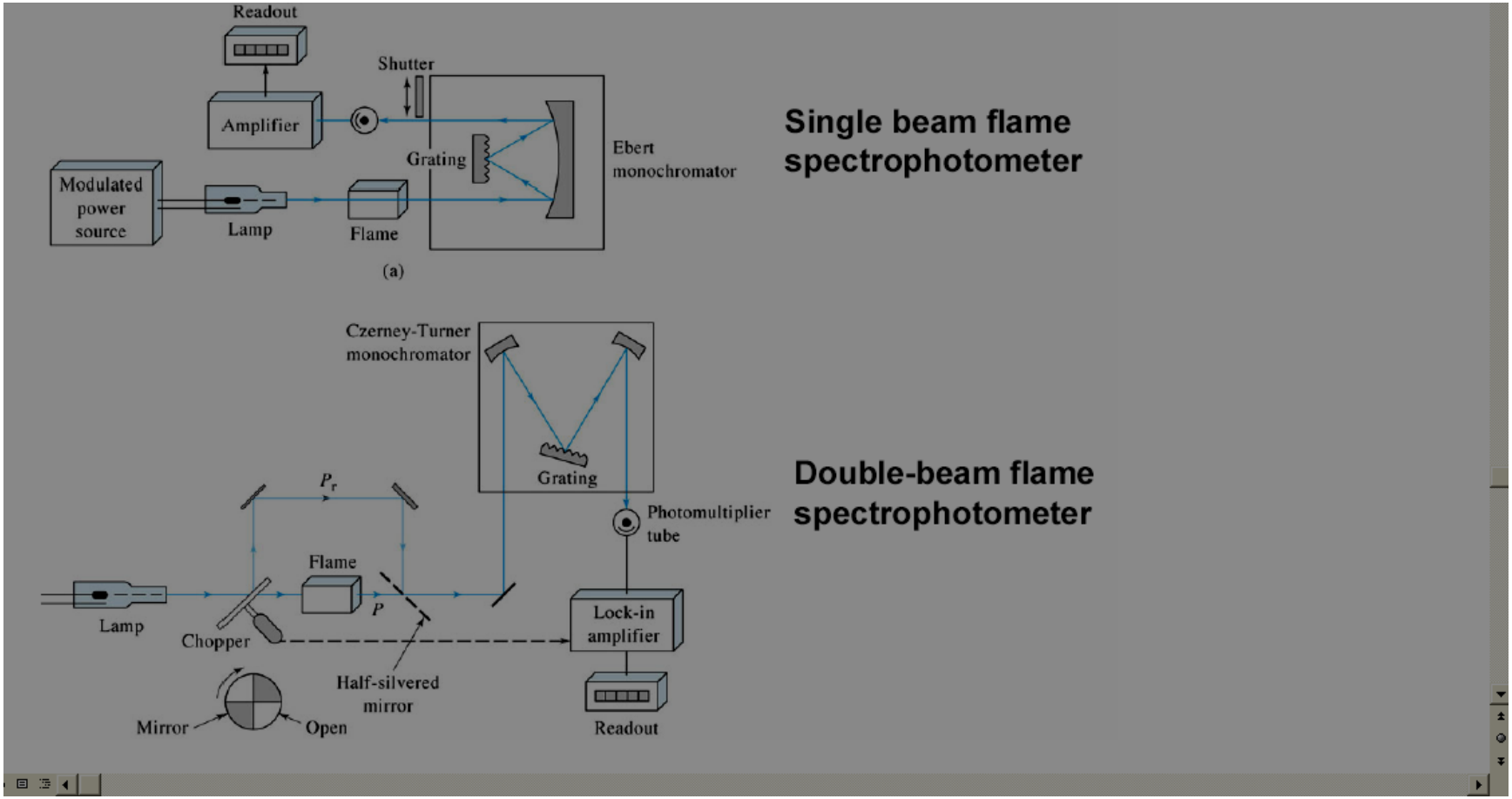
MONOCHROMATOR

- Some elements have single absorption line , but several elements have more than one absorption line .
- Hence it is necessary to select the spectral line for absorption have measurement.
- Moreover it is necessary to isolate the line spectrum of element from that of the emission by the gas in the lamp , or from the background signal of the flame.
- Hence a monochromator which can provide good resolution of 1nm or less is required

DETECTOR & READOUT DEVICE

- The intensity of radiation absorbed by elements, in UV or visible region (190-780nm) can be detected using photometric detectors.

- The readout device is capable of displaying the absorption spectrum as well as a specified wavelength.



Single beam flame spectrophotometer

Double-beam flame spectrophotometer

INTERFERENCE

- Spectral interference
- Chemical interference
- Ionic interference
- Matrix interference
- Solvent interference
- Dissociation of metal compound

APPLICATION OF AAS

- Estimation of trace elements in biological fluid like blood, urine, etc.
- Estimation of trace elements like Copper , Nickle and Zinc in food products.
- Estimation of Magnesium , Zinc in blood.
- Estimation of Zinc in Zinc insulin solution.
- Estimation of Mercury in Thiomersal solution.
- Estimation of Lead in Calcium carbonate and petrol.
- Estimation of elements in soil samples , water supply , effluents , ceramics , etc.