ESSENTIAL LAB TECHNIQUES FOR INDUSTRY

M.Sc. CHEMISTRY

SEMESTER-II, PAPER-III

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M.Sc. CHEMISTY: ESSENTIAL LAB TECHNIQUES FOR INDUSTRY

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FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A+' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the door step of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

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M.Sc. CHEMISTRY
SEMESTER-II, PAPER-III
203CH24 - ESSENTIAL LAB TECHNIQUES FOR INDUSTRY
SYLLABUS

Learning Objectives:

- To know the fundamentals in separation analysis using various chromatographic techniques.
- To know the techniques involving reliable separation by HPLC & amp; GC instrumental techniques.
- To know the purification by ion exchange chromatography.
- To know the instrumentation and applications of AAS & amp; ICP-OES.
- To know the basic principles, instrumentation and advantages UV, IR, NMR, ESR, TEM, SEM- techniques à structural analysis.

UNIT-I

Chromatography-Adsorption and Partition

1) **Introduction to Chromatography:** Different types of Chromatography. Adsorption chromatography- adsorbents, solvents, solutes, apparatus. Column Chromatography-stationary phase, Mobile phase, packing of column, advantages and disadvantages.
2) **Thin Layer Chromatography:** Basic Principles. Common stationary phases, Methods of preparing TLC plates, Selection of mobile phase, Development of TLC plates, Visualization methods, R value. Application of TLC in monitoring organic reactions.
3) **Paper Chromatography:** Basic Principles. Ascending and descending types. Selection of mobile phase, Development of chromatograms, Visualization methods. Application of paper chromatography in the identification of sugars and amino acids. One- and two-dimensional paper chromatography.

UNIT-II

High Performance Liquid Chromatography (HPLC): Basic Principles. Normal and reversed Phases. Selection of column and mobile phase. Instrumentation. detectors; RT values. Applications in the separation, identification and quantitative estimation of organic compounds. Concepts on HPLC method development.

UNIT-III

Gas Chromatography: Basic Principles. Different types of GC techniques. Selection of columns and carrier gases. Instrumentation. detectors; RT values. Applications in the separation, identification and quantitative analysis of organic compounds.

Ion Exchange Chromatography: Basic Principles. Preparation of cross-linked polystyrene resins. Different types of cation and anoin exchange resins. Application in the purification of carboxylic acids and amines.

UNIT-IV

AAS: Principle, instrumentation and applications

ICP-OES: Principle, instrumentation, applications and advantages over AAS.

UNIT-V

UV, IR, NMR, ESR, TEM, SEM-Basic principles, instrumentation and advantages.

Reference Books:

1) Principles of Instrumental Analysis by D.A. Skoog, F.J. Holler and T.A. Nieman, Harcourt College Pub.
2) Separation Techniques by M.N. Sastri, Himalaya Publishing House (HPH), Mumbai.
3) Bio Physical Chemistry by A. Upadhyay, K. Upadhyay and N. Nath, (HPH), Mumbai.
4) A Hand Book of Instrumental Techniques for Analytical Chemistry-Ed-F. A. Settle, Prearson Edn.,
5) Delhi. Introduction to Organic Laboratory Techniques - D.L. Pavia, G. M. Lampman, G.S. Kriz and R.G. Engel, Saunders College Pub. (NY).
6) Instrumental Methods of Chemical Analysis by B.K. Sharma, Goel Publish House, Meerut.
7) Instrumental Methods of Chemical Analysis by H. Kaur, Pragati Prakasan, Meerut.

Learning Outcomes:

- The student will understand advantage of cheromatographic separation and application on various reactions.
- The student will understand the advantage of HPLC & GC techniques over conventional separation techniques.
- The student will know the exchange of ions taking place in ion exchange chromatography.
- The student will know the procedure of analysing the elements using AAS & ICP-OES.
- The students understand the working principles and advantages of the UV, IR, NMR, ESR, TEM, SEM-techniques.

(203CH24)

MODEL QUESTION PAPER

M.Sc. DEGREE EXAMINATION,

CHEMISTRY - SECOND SEMESTER

ESSENTIAL LAB TECHNIQUES FOR INDUSTRY

Time: Three Hours **Maximum: 70 Marks**

Answer all the following questions

UNIT-I

1) a) Define adsorption chromatography. Mention any two common adsorbents and two uses of column chromatography **[4]**

OR

b) What is Rf value in TLC? How is TLC used to monitor the completion of an organic reaction?

2) a) Describe the principle, apparatus, packing, advantages and disadvantages of column chromatography. **[10]**

OR

b) Explain the principles and procedure of TLC and paper chromatography.

UNIT-II

3) a) What is the basic principle of HPLC? Distinguish between normal-phase and reversed-phase HPLC **[4]**

OR

b) What is retention time (RT) in HPLC? How is it used for identification of compounds?

4) a) Describe the instrumentation of an HPLC system and the selection of column and mobile phase **[10]**

OR

b) Explain, step by step, how you would develop and validate an HPLC method for a single organic compound in a mixture

UNIT-III

5) a) Why must the sample be volatile in GC? Give two examples of GC detectors **[4]**

OR

b) What is meant by capacity of an ion exchange resin? How does cross linking affect it?

6) a) Explain the principle, instrumentation and applications of gas chromatography in the analysis of organic compounds **[10]**

OR

b) Describe the preparation of cross-linked polystyrene ion exchange resins and explain how ion exchange chromatography is used to purify carboxylic acids and amines.

UNIT-IV

7) a) What is a hollow cathode lamp and why is it used in AAS? **[4]**

OR

b) Define detection limit. Why is ICP OES more sensitive than flame AAS?

8) a) Describe flame AAS and graphite furnace AAS, comparing sensitivity and applications **[10]**

OR

b) Explain the principle of ICP OES, the role of plasma, and how emission lines are used for multi element analysis

UNIT-V

9) a) What is the difference between UV–Vis absorption and IR absorption in terms of molecular transitions? **[4]**

OR

b) State the basic idea of chemical shift in NMR with one simple example.

10) a) Discuss how UV, IR and NMR together can be used to elucidate the structure of an unknown organic compound. **[10]**

OR

b) Explain the principles of TEM and SEM and compare their use for observing the morphology and structure of solid samples.

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LESSON-1

CHROMATOGRAPHY-ADSORPTION AND PARTITION

1.0 OBJECTIVES:

After studying this lesson, you should be able to:

- To know about the Chromatography.
- To study about Different Types of Chromatography
- To study about Partition Chromatography and Adsorption Chromatography
- To study about Some of terms involved in Chromatography

STRUCTURE:

1.1 Introduction to Chromatography
1.2 Different Types of Chromatography
1.3 Different Forms of Chromatography
1.4 Some of Terms Involved in Chromatography
1.5 Partition Chromatography
1.6 Adsorption Chromatography
1.7 Summary
1.8 Self-Assessment Questions
1.9 Reference Books

1.1. INTRODUCTION TO CHROMATOGRAPHY:

Chromatography is a method used by scientists for separating organic and inorganic compounds so that they can be analyzed and studied. By analyzing a compound, a scientist can figure out what makes up that compound. Chromatography is a great physical method for observing mixtures and solvents. The word chromatography means "color writing" which is a way that a chemist can test liquid mixtures. While studying the coloring materials in plant life, M.S. Tswett a Russian botanist invented chromatography in 1903. His name was.

The power of chromatography comes from its ability to separate a mixture of compounds, or “analytes”, and determine their respective identity (chemical structure) and concentration.

Chromatography can be divided into three basic types that include gas, liquid, and supercritical fluid chromatography (**Figure 1.1**).

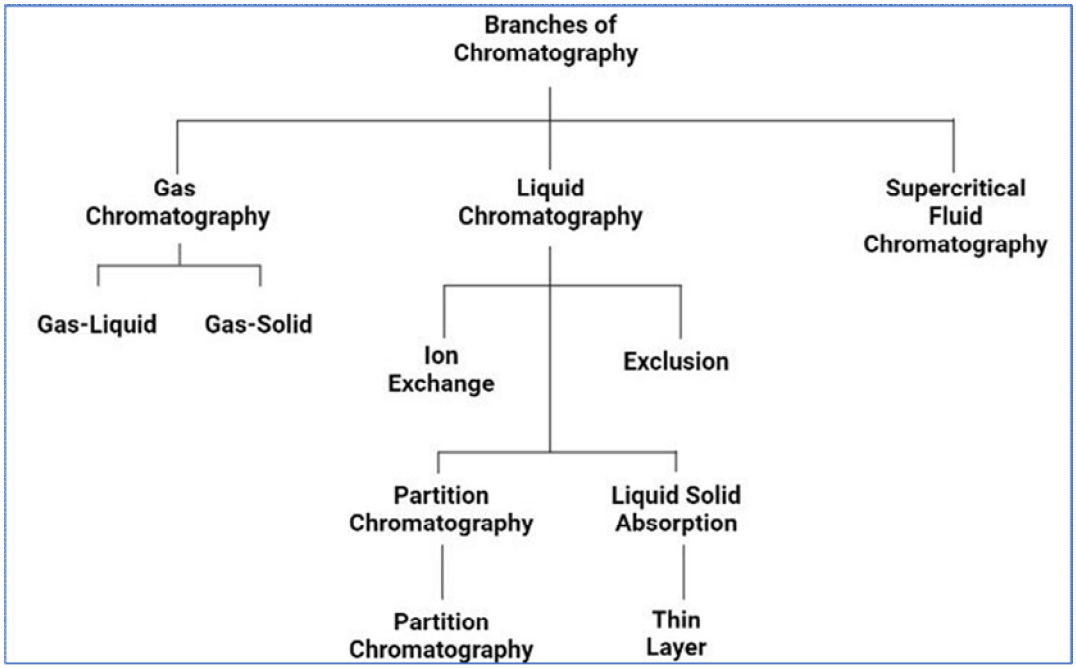

Figure 1.1: Categories of Chromatography

In general, each type of chromatography is comprised of two distinct steps: chromatography (or separation of individual compounds in distinct elution bands) and identification (detection of each elution band).

- Gas chromatography is the process of taking a sample and injecting it into the instrument, turning the solvent and analytes into gaseous form, and separating the mixture of compounds into individual peaks (and preferably individual compounds).
- Liquid chromatography completes the same process except the separations occur in a liquid phase. Individual band or peaks exit the column and identification occurs by a relatively universal detector.

One particularly common detector for both gas and liquid chromatography is mass spectrometry (MS) which transforms each analyte from a chemically neutral species into a positive cation, usually breaking various bonds in the process. Detecting the mass of the individual pieces (referred to as fragments) allows for conclusive identification of the chemical structure of the analyte.

1.2. DIFFERENT TYPES OF CHROMATOGRAPHY:

There are four main types of chromatography. These are Liquid Chromatography, Gas Chromatography, Thin-Layer Chromatography and Paper Chromatography (**Figure 1.2**).

Liquid Chromatography is used in the world to test water samples to look for pollution in lakes and rivers. It is used to analyze metal ions and organic compounds in solutions. Liquid chromatography uses liquids which may incorporate hydrophilic, insoluble molecules.

Gas Chromatography is used in airports to detect bombs and is used is forensics in many ways. It is used to analyse fibers on a person's body and also analyze blood found at a crime scene. In gas chromatography helium is used to move a gaseous mixture through a column of absorbent mate rial.

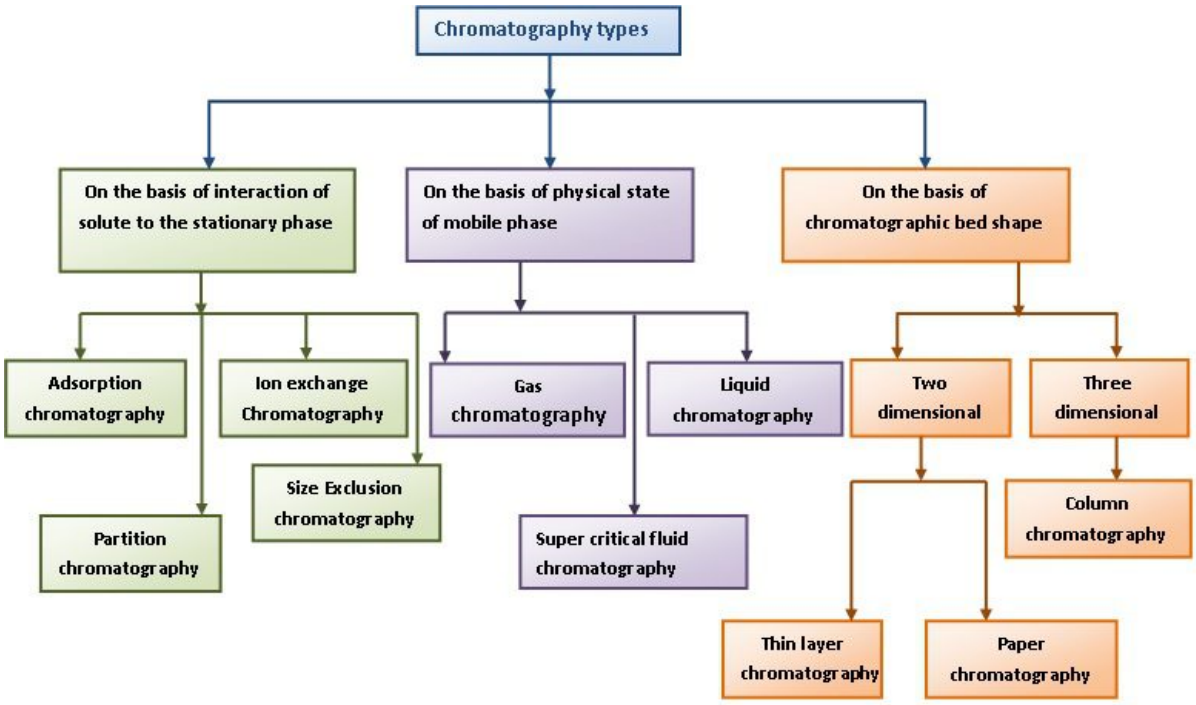

Figure 1.2: Types of Chromatography

Thin-layer Chromatography uses an absorbent material on flat glass or plastic plates. This is a simple and rapid method to check the purity of an organic compound. It is used to detect pesticide or insecticide residues in food. Thin-layer chromatography is also used in forensics to analyze the dye composition of fibers.

Paper Chromatography is one of the most common types of chromatography. It uses a strip of paper as the stationary phase. Capillary action is used to pull the solvents up through the paper and separate the solutes.

1.3. DIFFERENT FORMS OF CHROMATOGRAPHY

***Partition Chromatography*:**

In this form of chromatography, an analyte distributes themselves into two phases, liquid stationary, and mobile phase. The major advantage of this chromatography is that it is simple, low cost and has broad specificity. It is further divided into liquid-liquid chromatography and bonded-phase liquid chromatography. The example of this chromatography is cellulose, starch, or silica matrix (**Figure 1.3 and Table 1.1 & 1.2)**.

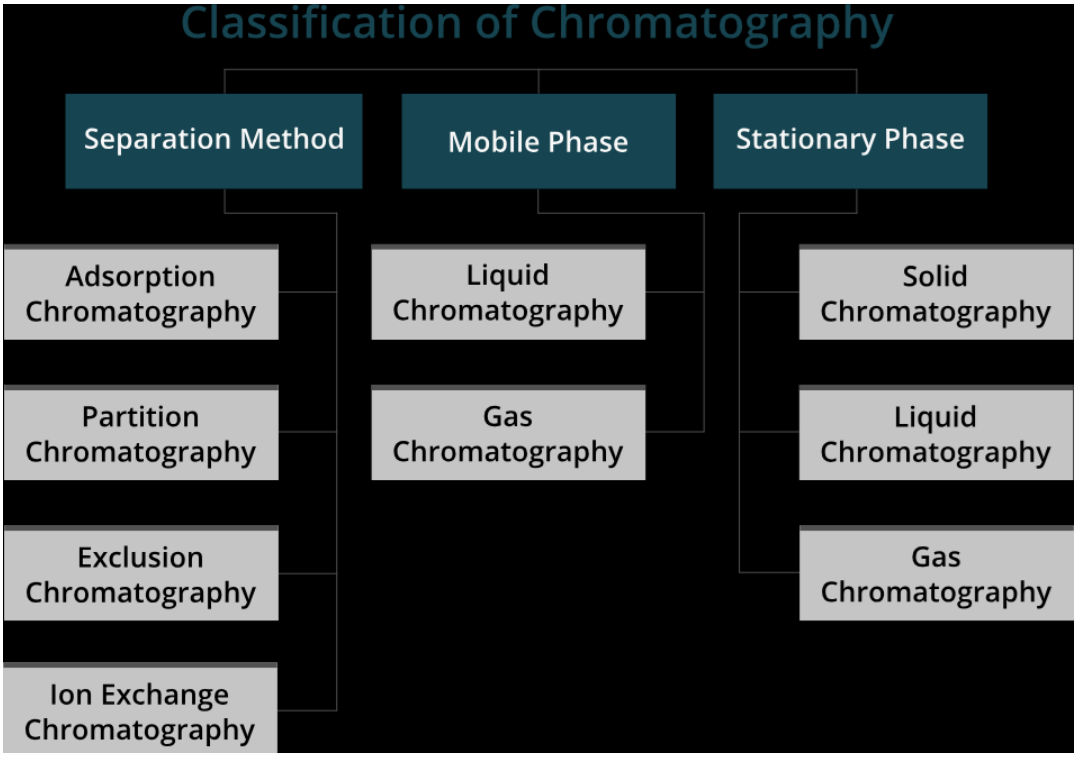

Figure 1.3: Classification of Chromatography

***Adsorption Chromatography*:**

In this form of chromatography, matrix molecule has ability to hold the analyte on their surface through a mutual interaction due to different types of forces such as hydrogen bonding, electrostatic interaction, vander waal etc. The example is ion-exchange chromatography, hydrophobic interaction chromatography, affinity chromatography etc.

Table 1.1: Chromatographic Techniques with Stationary Phase and Mobile Phase

Type of Chromatography	Mobile Phase	Stationary Phase	Stationary Phase Support	Technique	Acronym
Adsorption chromatography	Gas	Solid	Column	Gas-solid chromatography	GC/GSC
	Liquid	Solid	Column	Liquid column chromatography	LC
				High performance liquid chromatography	HPLC
		Solid	Planar layer	Thin layer chromatography	TLC
Partition chromatography	Gas	Liquid	Column	Gas-liquid chromatography	GC/GLC
	Liquid	Liquid	Column	Liquid-liquid chromatography	LC
				High performance liquid chromatography	HPLC
		Liquid	Planar layer	Paper chromatography	PC
Ion exchange chromatography	Liquid	Exchange resin	Column	Ion exchange chromatography	IEX
Permeation chromatography	Liquid	Polymer matrix	Column	Size exclusion chromatography	SEC/GPC

Table: 2. Chromatographic techniques with mobile and stationary phase.

Mobile phase	Stationary phase	Technique
Liquid	Liquid	Partition chromatography
Gas	Liquid	Gas chromatography
Liquid	Ion exchange resin	Ion exchange chromatography
Liquid	Molecular sieves	Gel permeation, ion exclusive
Liquid	Thin layer of silica or alumina	Thin layer chromatography
Liquid	Paper	Paper chromatography

1.4. SOME OF TERMS INVOLVED IN CHROMATOGRAPHY:

Chromatography:

A physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a specific direction

Chromatogram:

A graphical or other presentation of detector response, concentration of analyte in the effluent or other quantity used as a measure of effluent concentration versus effluent volume or time

Solvents:

It is also termed as eluting agent. Solvents are used either to transfer the mixture of the column or for bringing about the process of desorption which results in the separation of the components into bands and finally their elutions. Solvent may be the same throughout or it may be different at different stages and for different components of the mixture.

In choosing eluting agent two considerations are taken into account.

1) Solvent should satisfy the practical factors such as viscosity, stability, comparability with detection, solubility with respect to the sample, suitable purity etc.
2) Solvent should provide maximum resolution for the separation of sample in reasonable time.

In general, it is suggested that adsorption effects are strongest in non-polar media. It is therefore a usual practice to apply the mixture to be separated as a solution in a non-polar solvent such as petroleum ether, benzene etc.

For the development of the chromatogram, a slightly more polar solvent may be used, so that the more weakly held components are displaced and moved down the column.

As a rough guide to the sequence of solvents used in elution of lipophilic materials, an *'Eluotropic series'* has been suggested. The order is as follows: petroleum ether, cyclohexane, benzene, chloroform, acetone, ethyl acetate, ethanol, methanol, water.

Mobile Phase:

A fluid which flows through or along the stationary phase, in a specific direction. It may be a liquid (liquid chromatography) or a gas (gas chromatography) or a supercritical fluid (supercritical-fluid chromatography).

Stationary Phase:

One of the two phases forming a chromatographic system. It may be a solid, a gel or a liquid. If a liquid, it may be distributed on a solid. This solid may or may not contribute to the separation process. The liquid may also be chemically bonded to the solid (bonded phase: covalently bonded to the support particles or to the inside wall of the column tubing) or immobilized onto it (immmobilized phase)

Contaminant:

A contaminant is an impurity arising from an unintended source. Contaminants might include ligand leakage from an affinity matrix, endotoxins from unwanted bacterial infection, viruses, or other materials introduced due to the bioburden.

Effluent:

The effluent is the mobile phase (eluent) that leaves the chromatography column or device.

Eluent:

The eluent is the mobile phase that flows through a chromatography matrix. It is frequently used to describe the mobile phase at any stage in chromatography, whether entering, passing through, or leaving the matrix during sample application or desorption (elution).

Elution:

Elution is the flow of eluent through a chromatography matrix. It is often used to describe the desorption phase when the target is recovered in bind & elute mode.

1.5. PARTITION CHROMATOGRAPHY:

When a solute is shaken with two immiscible solvents it will distribute itself unevenly between the two phases and at equilibrium, the ratio of concentration of the compound in two solvents is constant and is known as partition coefficient.

$$K = \frac{\text{Concentration of 'X' in solevnt (1)}}{\text{Concentration of 'X' in Solvent (2)}}$$

K is the partition coefficient, and will be constant for a given temperature.

In partition chromatography, one of the two solvents is held on a column as the stationary phase. Silica gel is probably the most useful material for making up the column

since it can bind upto about 5% of its own weight of water without becoming physically wet. This means that a free-running powder can be packed in a chromatography tube into a column in which the silica gel functions simply as an inert support for the stationary phase of water.

When performing a chromatographic separation of a mixture by this technique, the principle of the method depends on the partition of components of the mixture between water held on the column and non-polar solvent percolating down the column. Thus, the more hydrophilic components, having a marked affinity for water, will be held at the top of the column, whilst the more hydrophobic (or Lipophilic) materials, being soluble in non-polar medium, will move down the column and will therefore be eluted first.

A stationary phase of water is quite suitable when the compounds to be separated have sufficient of a hydrophilic character; but highly non-polar compounds have too little affinity for such a column to allow proper separation. In order to overcome this difficulty, a method of Reversed – phase Chromatography was introduced later, to provide a hydrophobic phase. Hydrophobic materials can also be used on column to separate hydrophobic compounds. So, it is called *Reverse Phase Partition Chromatography*. Ex: Dimethyl dichloro silane.

Applications:

1) Partition Chromatography has become a powerful tool for the separation of closely related substances such as numerous amino acids formed in the hydrolysis of protein, separations and analysis of closely related aliphatic alcohols and separation of sugar derivatives.
2) Partition Chromatography is useful in the separation of organic dibasic acids as described hereunder.

1.6. ADSORPTION CHROMATOGRAPHY:

The technique in which the stationary phase is solid (ex: alumina or silica gel) and the mobile phase is either a gas or a liquid is known as Adsorption chromatography. Since adsorption is a surface phenomenon, the degree of separation depends upon the surface area of the adsorbent.

Principle:

The basic principle is adsorption, and can be fairly specific so that one solute may be adsorbed selectively from a mixture. Separation of components depends upon differences both in their degree of adsorption by the adsorbent and solubility in the solvent and these two are in turn governed by molecular structure of compound.

Adsorbent:

Many adsorbents are available, and commonly used materials are given below.

1) Aluminium oxides (acidic, basic and neutral grades)
2) Bentonite,
3) Calcium carbonate,
4) Calcium hydroxide,

5) Calcium phosphate,
6) Charcoal,
7) Florisil (a synthetic material based on magnesia and silica gel)
8) Fuller's earth
9) Kieselguhr
10) $MgCO_3$ etc.

Adsorbents Activity:

Adsorbents have been classified into grades of activity as weak, medium, or strong depending on their power of adsorption. It is also possible to activate or deactivate the adsorbent. Some materials are activated by heating or pretreatment with acid or bases (as with alumina) or organic solvent (ex: charcoal).

Deactivation of alumina has been affected by the addition of water and the different grades of alumina thus produced are standardized according to *Brockmann Activity* which is based on the adsorption capacity of various dyes.

Applications: The technique is useful

1) in separation of Methyl Blue and Fluoresce in (sodium salt)
2) in separation of 2,4 Dinitrophenyl hydrozones.
3) in separation of mixture containing sterioisomers or related compounds.
4) Separation of the ordinary 17- keto-steroids.
5) Separation of plasma cortisol

1.7. SUMMARY:

1) To know about the Chromatography.
2) To study about Different Types of Chromatography
3) To study about Partition Chromatography and Adsorption Chromatography
4) To study about Some of terms involved in Chromatography

1.8. SELF-ASSESSMENT QUESTIONS:

1) Differentiate adsorption and partition chromatography.
2) Write the various types of chromatography.
3) Define mobile phase and stationary phase.
4) What is elution and eluent.

1.9. REFERENCE BOOKS:

1) Principles of Instrumental Analysis by D.A. Skoog, F.J. Holler and T.A. Nieman, Harcourt College Pub.

2) Separation Techniques by M.N. Sastri, Himalaya Publishing House (HPH), Mumbai.

3) A Hand Book of Instrumental Techniques for Analytical Chemistry - Ed-F. A. Settle, Prearson Edn.

Dr. K. Bala Murali Krishna

LESSON-2

COLUMN CHROMATOGRAPHY

2.0. OBJECTIVES:

After studying this lesson, you should be able to:

- To know about the Column Chromatography.
- To study about Different Types of Column Chromatography and Basic Principle.
- To know about Packing of column.
- To study about Column Chromatography procedure and experiment.
- To learn about applications, advantages, disadvantages of Column Chromatography.

STRUCTURE:

2.1 Introduction to Column Chromatography

2.2 Types of Column Chromatography

2.3 Column Chromatography Principle

2.4 Column Chromatography Procedure

2.5 Chromatography Columns

2.6 Packing the Column

2.7 Column Chromatography Experiment

2.8 Applications of Column Chromatography

2.9 Advantages of Column Chromatography

2.10 Disadvantages of Column Chromatography

2.11 Summary

2.12 Self-Assessment Questions

2.13 Reference Books

2.1. INTRODUCTION TO COLUMN CHROMATOGRAPHY

Column chromatography is a technique which is used to separate a single chemical compound from a mixture dissolved in a fluid. It separates substances based on differential adsorption of compounds to the adsorbent as the compounds move through the column at different rates which allow them to get separated in fractions. This technique can be used on small scale as well as large scale to purify materials that can be used in future experiments. This method is a type of adsorption chromatography technique.

2.2. TYPES OF COLUMN CHROMATOGRAPHY:

1) Adsorption column chromatography – Adsorption chromatography is a technique of separation, in which the components of the mixture are adsorbed on the surface of the adsorbent.
2) Partition column chromatography – The stationary phase, as well as mobile phase, are liquid in partition chromatography.
3) Gel column chromatography – In this method of chromatography, the separation takes place through a column packed with gel. The stationary phase is a solvent held in the gap of a solvent.
4) Ion exchange column chromatography – A chromatography technique in which the stationary phase is always ion exchange resin.

2.3. COLUMN CHROMATOGRAPHY PRINCIPLE:

When the mobile phase along with the mixture that needs to be separated is introduced from the top of the column, the movement of the individual components of the mixture is at different rates. The components with lower adsorption and affinity to stationary phase travel faster when compared to the greater adsorption and affinity with the stationary phase. The components that move fast are removed first whereas the components that move slow are eluted out last.

The adsorption of solute molecules to the column occurs in a reversible manner. The rate of the movement of the components is expressed as:

$$\text{Rf} = \frac{\text{Distance traveeled by the solute}}{\text{Distance traveeled by the solvent}}$$

Rf is the retardation factor.

2.4. COLUMN CHROMATOGRAPHY PROCEDURE:

Before starting with the Column Chromatography Experiment let us understand the different phases involved.

Mobile Phase: This phase is made up of solvents and it performs the following functions:

1) It acts as a solvent – sample mixture can be introduced in the column.
2) It acts as a developing agent – helps in the separation of components in the sample to form bands.
3) It acts as an eluting agent – the components that are separated during the experiment are removed from the column
4) Some examples of solvents used as mobile phase based on their polarity are – ethanol, acetone, water, acetic acid, pyridine, etc.

Stationary phase: It is a solid material which should have good adsorption property and meet the conditions given below:

1) Shape and size of particle: Particles should have uniform shape and size in the range of 60 – 200μ in diameter.

2) Stability and inertness of particles: high mechanical stability and chemically inert. Also, no reaction with acids or bases or any other solvents used during the experiment.
3) It should be colourless, inexpensive and readily available.
4) Should allow free flow of mobile phase
5) It should be suitable for the separation of mixtures of various compounds.

2.5. CHROMATOGRAPHY COLUMNS:

The basic equipment usually consists of a long vertical glass tube, restricted (constricted) at the lower end, in which column the packing material is contained as the Stationary Phase. It is important that the stationary phase consists of uniform particles, packed uniformly, air bubbles must be absent and the packing must never be allowed to become dry, that is solvent must always be present above the level of the stationary support (**Figure 2.1**).

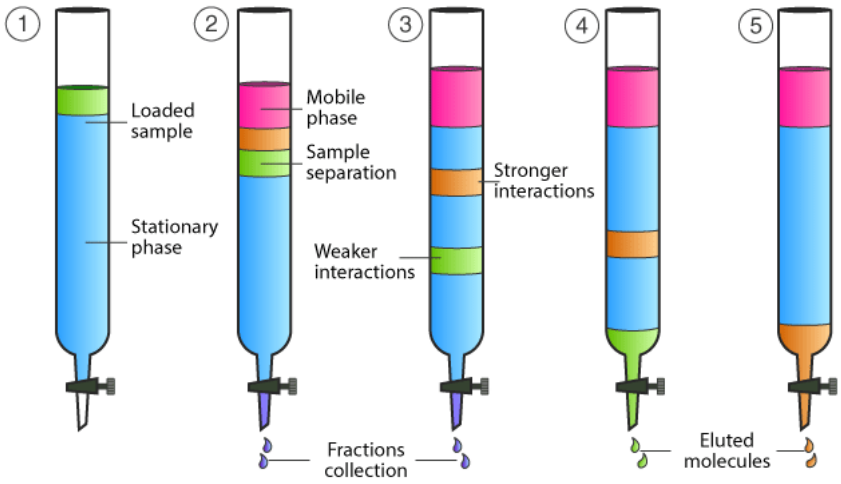

Figure 2.1: Representation of Chromatographic Separation Using Column

2.6. PACKING THE COLUMN:

The inert packing that is to form the stationary support of the column should consist of uniform particles, and these are usually graded by sieving or less frequently, by decantation of fine particles from a slurry. Fortunately, such materials are commercially available in each range of particle size 100-200 mesh, 200-300 mesh etc.

In general, the most suitable particle is chosen by a balance between two factors. Thus, the smaller the particle size, the better will be the separation of components, but the larger the particles, the better flow rate. Packing of 100-200 mesh will normally be quite suitable for simple separation.

It is occasionally possible to pack the column with stationary phase in dry form, and then to add the solvent, but this is liable to the introduction of air bubbles which cannot be removed.

The most favoured method of packing a column is to prepare slurry with the dry packing in the minimum amount of solvent that allows a free running mixture. The slurry is poured in one movement into the chromatographic column, and therefore settles uniformly. Small amounts of solvent may then be added to rinse the sides of the tube, which is tapped occasionally to aid sedimentation, and the liberation of air bubbles.

Final Preparation of Column:

The technique of column chromatography involves the application of a mixture at the top of the column in as compact a form as possible, followed by a separation of components brought about by a solvent percolating down the stationary support. In the simplest case, it is convenient to add the solvent from a tap funnel that is connected to the top of the column by an airtight joint. This may be simply a rubber bung, or perhaps a ground glass joint. In view of the disturbance of the packing that would normally occur from the drip of the solvent, it is usual to float a disk on top of solvent column. The disk may be a cork, though polythene is more inert. Alternatively, another plug of glass wool may be inserted above the packing to protect it from disturbance. It has been already stated that positive or negative pressure may be applied to the column to boast the flow rate and that positive pressure is to be preferred. On the other hand, it is more convenient to apply suction to the base of the column, and this may be affected by connecting to the Buchner Flask **(Figure 2.2**).

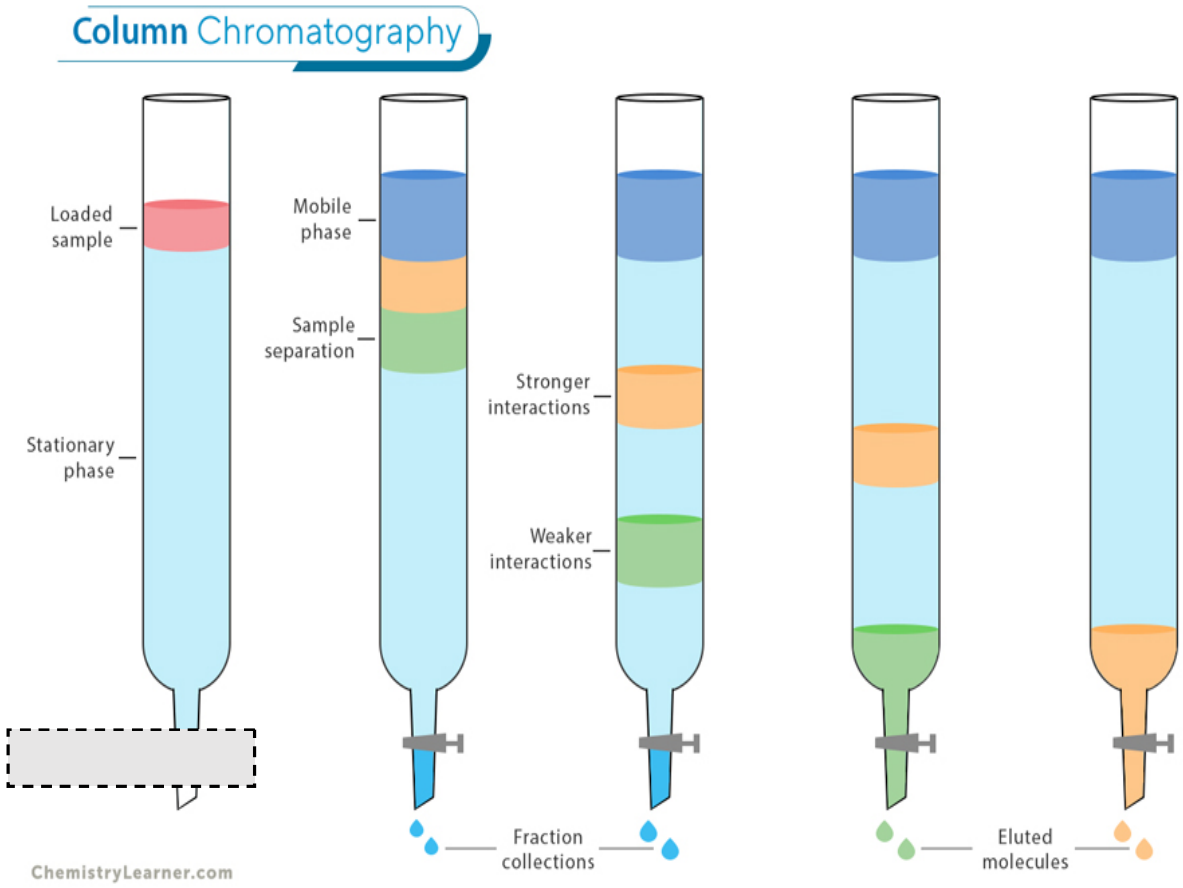

Figure 2.2: Representation of Column Preparation with their Eluents

2.7. COLUMN CHROMATOGRAPHY EXPERIMENT:

The stationary phase is made wet with the help of solvent as the upper level of the mobile phase and the stationary phase should match. The mobile phase or eluent is either solvent or mixture of solvents. In the first step the compound mixture that needs to be separated, is added from the top of the column without disturbing the top level. The tap is turned on and the adsorption process on the surface of silica begins (**Figure 2.3**).

Without disturbing the stationary phase solvent mixture is added slowly by touching the sides of the glass column. The solvent is added throughout the experiment as per the requirement.

The tap is turned on to initiate the movement of compounds in the mixture. The movement is based on the polarity of molecules in the sample. The non-polar components move at a greater speed when compared to the polar components.

For example, a compound mixture consists of three different compounds viz red, blue, green then their order based on polarity will be as follows

blue > red > green.

As the polarity of the green compound is less, it will move first. When it arrives at the end of the column it is collected in a clean test tube. After this, the red compound is collected and at last blue compound is collected. All these are collected in separate test tubes.

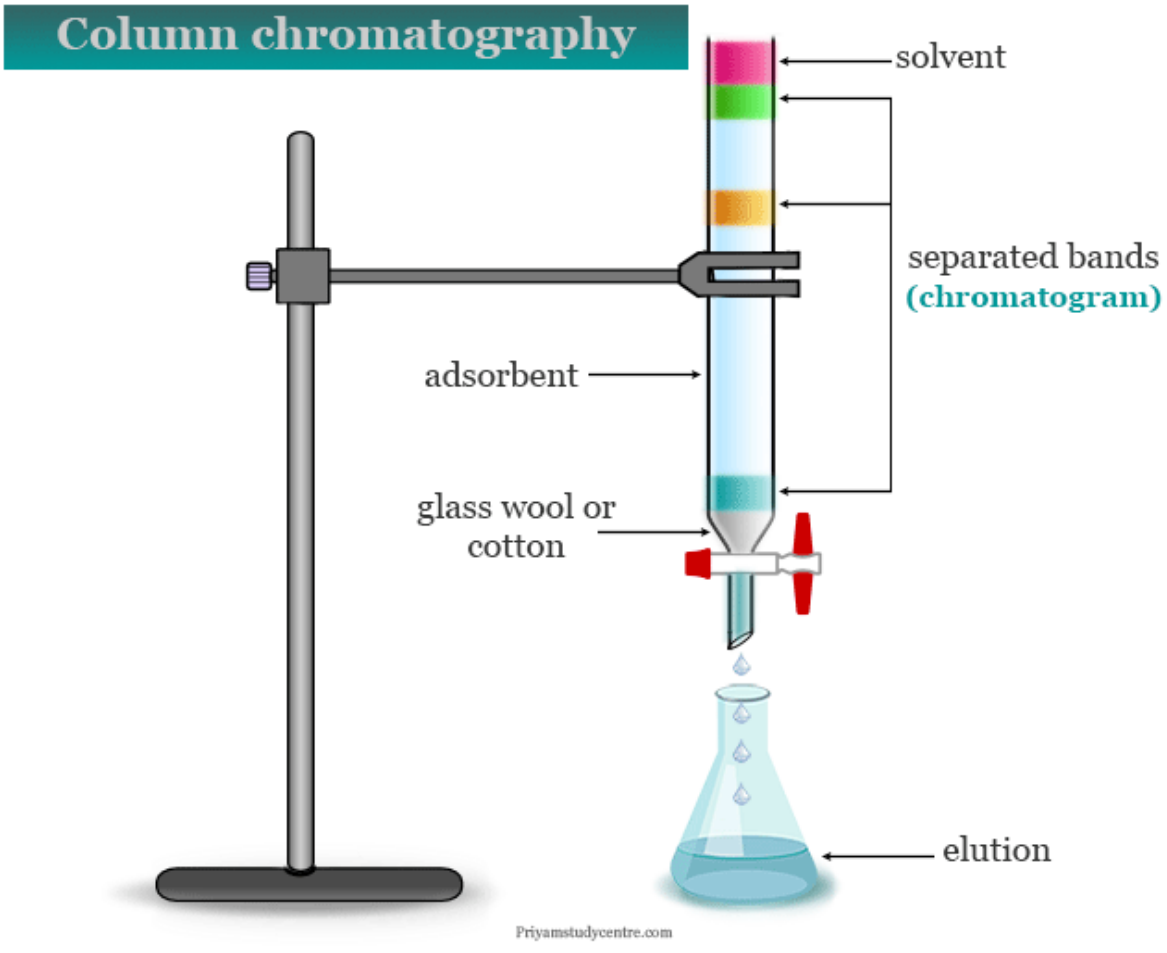

Figure 2.3: Schematic Representation of Column Experiment

2.8. APPLICATIONS OF COLUMN CHROMATOGRAPHY:

- Column Chromatography is used to isolate active ingredients.
- It is very helpful in Separating compound mixtures.
- It is used to determine drug estimation from drug formulations
- It is used to remove impurities.
- Used to isolation metabolites from biological fluids.

2.9. ADVANTAGES OF COLUMN CHROMATOGRAPHY:

- It facilitates the separation of any mixture.
- It aids in the removal of impurities from any type of mixture.
- This allows for the separation of any variety and quantity.
- Depending on the desired results, different solvents can also be used in the separation process.
- The procedure can also be automated.
- It is less costly.

2.10. DISADVANTAGES OF COLUMN CHROMATOGRAPHY

- It is a lengthy procedure.
- Separation requires a large amount of material.
- It is more expensive than thin paper column chromatography.
- It's a lengthy procedure. As a result, focus and attention are required.
- It will become more expensive if the process is automated in the future.
- Time-Consuming: The separation process can take a long time, depending on the complexity of the mixture.
- Large Solvent Requirement: Requires a large volume of solvents for elution.
- Manual Operation: The process is mostly manual, requiring careful monitoring.

2.11. SUMMARY:

- To know about the Column Chromatography.
- To study about Different Types of Column Chromatography and Basic Principle.
- To know about Packing of column.
- To study about Column Chromatography procedure and experiment.
- To learn about applications, advantages, disadvantages OF Column Chromatography.

2.12. SELF-ASSESSMENT QUESTIONS:

1) Discuss about column chromatography in detail.
2) Write the selection of mobile phase and stationary phase in column chromatography.
3) Explain in detail on packing of column.
4) What are the principle involved in column chromatography and write advantages and disadvantages of column chromatography.

2.13. REFERENCE BOOKS:

1) Principles of Instrumental Analysis by D.A. Skoog, F.J. Holler and T.A. Nieman, Harcourt College Pub.
2) Separation Techniques by M.N. Sastri, Himalaya Publishing House (HPH), Mumbai.
3) A Hand Book of Instrumental Techniques for Analytical Chemistry- Ed-F.A. Settle, Prearson Edn.

Dr. K. Bala Murali Krishna

LESSON-3

THIN LAYER CHROMATOGRAPHY

3.0. OBJECTIVES:

After studying this lesson, you should be able to:

- To know about the Basic Principle in involved in thin layer Chromatography.
- To study about the selection of Stationary and Mobile phases.
- To know about Development and Spotting of TLC plates.
- To study about methods of preparing TLC plates.
- To known the importance of R_f value.
- To know about Visualization methods for TLC.
- To study the applications, advantages, disadvantages of TLC.

STRUCTURE:

3.1 Introduction to Thin Layer Chromatography (TLC)

3.2 Thin Layer Chromatography Principle

3.3 Common Stationary Phases (TLC Plate)

3.4 Selection of Mobile Phase (Solvent)

3.5 Methods of preparing TLC plates

3.6 Development and Spotting of TLC plates

3.7 Thin Layer Chromatography Procedure

3.8 Thin Layer Chromatography Experiment

3.9 Visualization of TLC Spots

3.10 Retention Factor (R_f)

3.11 Thin Layer Chromatography Applications

3.12 Thin Layer Chromatography Advantages

3.13 Thin Layer Chromatography Disadvantages

3.14 Summary

3.15 Self-Assessment Questions

3.16 Reference Books

3.1. INTRODUCTION TO THIN LAYER CHROMATOGRAPHY (TLC):

TLC as a procedure for analytical chromatography was first introduced by Stahl (1958). He was mainly responsible for bringing out standard equipment for preparing thin layers. TLC is often named by other names such as drop, strip, spread layer, surface chromatography or open column chromatography.

Thin Layer Chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminium foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminium oxide, cellulose, or silica gel.

3.2. THIN LAYER CHROMATOGRAPHY PRINCIPLE:

Like other chromatographic techniques, thin-layer chromatography (TLC) depends on the separation principle. The separation relies on the relative affinity of compounds towards both the phases. The compounds in the mobile phase move over the surface of the stationary phase. The movement occurs in such a way that the compounds which have a higher affinity to the stationary phase move slowly while the other compounds travel fast. Therefore, the separation of the mixture is attained. On completion of the separation process, the individual components from the mixture appear as spots at respective levels on the plates. Their character and nature are identified by suitable detection techniques (**Figure 3.1**).

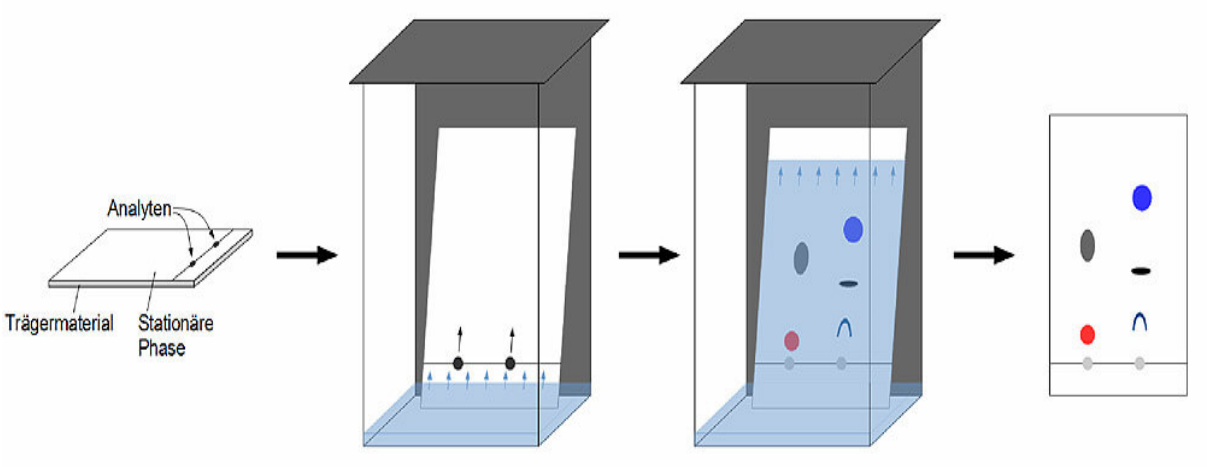

Figure 3.1: TLC Plate in TLC Chamber

3.3. COMMON STATIONARY PHASES (TLC PLATE):

Silica gel and alumina are among the most common stationary phases, but others are available as well. Many plates incorporate a compound which fluoresces under short-wave UV (254 nm). The backing of TLC plates is often composed of glass, aluminum, or plastic**.**

Coating Material:

Many coating materials are known which are commercially produced for use as thin layer adsorbent (**Table 3.1**).

Table 3.1: Coating Materials used in TLC

Adsorbent	Acidic/Basic	Activity	Separating Mechanism	Components to be separated
Silica gel	acidic	Active	Adsorption/ Partition	Acidic & neutral substances
Alumina	basic	Active	Adsorption/ Partition	Basic & neutral substances
Kieselguhr	neutral	Inactive	Partition	Strongly hydrophilic substances
Cellulose	neutral	None	Partition	Water soluble compounds

Glass plates are chemically inert and best withstand reactive stains and heat, but are brittle and can be difficult to cut. Aluminium and plastic plates can be cut with scissors, but aluminium may not withstand strongly acidic or oxidizing stains, and plastic does not withstand the high heat required to develop many stains. Aluminium and plastic plates are also flexible, which may result in flaking of the stationary phase. Never under any circumstances touch the face of a TLC plate with your fingers as contamination from skin oils or residues on gloves can obscure results. Instead, always handle them by the edges, or with forceps.

3.4. SELECTION OF MOBILE PHASE (SOLVENT):

Proper solvent selection is perhaps the most important aspect of TLC, and determining the best solvent may require a degree of trial and error. As with plate selection, keep in mind the chemical properties of the analytes. A common starting solvent is 1:1 hexane:ethyl acetate. Varying the ratio can have a pronounced effect of R_f.

R_f values range from 0 to 1 with 0 indicating that the solvent polarity is very low and 1 indicating that the solvent polarity is very high. When performing your experiment, you do not want your values to be 0 or 1 because your components that you are separating have different polarities. If the value is 0, you need to increase your solvent polarity because the sample is not moving and sticking to the stationary phase. If the value is 1, you need to decrease your solvent polarity because the compound could not separate.

If you know that one component of a mixture is insoluble in each solvent, but another component is freely soluble in it, it often gives good separations. How fast the compounds travel up the plate depends on two things:

- If the compound is soluble in the solvent, it will travel further up the TLC plate.
- How well the compound likes the stationary phase. If the compound likes the stationary phase, it will stick to it, which will cause it to not move very far on the chromatogram.

The volatility of solvents should also be considered when chemical stains are to be used. Any solvent left on the plate may react with the stain and conceal spots. Many solvents can be removed by allowing them to sit on the bench for a few minutes, but very non-volatile

solvents may require time in a vacuum chamber. Volatile solvents should only be used once. If the mobile phase is used repeatedly, results will not be consistent or reproducible.

Useful Solvent Mixtures:

- A solvent that can be used for separating mixtures of strongly polar compounds is ethyl acetate: butanol: acetic acid: water, 80:10:5:5.
- To separate strongly basic components, make a mixture of 10% NH_4OH in methanol, and then make a 1 to 10% mixture of this in dichlormethane (DCM).
- Mixtures of 10% methanol or less in DCM can be useful for separating polar compounds.
- Benzene-ethanol (9:1), Chloroform, Benzene, Cyclohexane, High boiling Paraffins, Ethyl acetate,
- Ethyl acetate - methanol (99:1), Benzene- methanol (95:5) are used as solvents in TLC.

3.5. METHODS OF PREPARING TLC PLATES:

TLC plates are usually commercially available, with standard particle size ranges to improve reproducibility. They are prepared by mixing the adsorbent, such as silica gel, with a small amount of inert binder like calcium sulfate (gypsum) and water. This mixture is spread as a thick slurry on an unreactive carrier sheet, usually glass, thick aluminum foil, or plastic. The resultant plate is dried and activated by heating in an oven for thirty minutes at 110 °C. The thickness of the absorbent layer is typically around 0.1–0.25 mm for analytical purposes and around 0.5–2.0 mm for preparative TLC. As the chemicals being separated may be colorless, several methods exist to visualize the spots.

Many applicators are commercially available which are used for coating the glass plates with different adsorbents layers of uniform thickness. Most of these methods use the coating materials in the form of suspension or slurry in some liquid. The various methods of preparing layers are as follows (**Figure 3.2**)

1) Pouring
2) Dipping
3) Spraying
4) Spreading

1) **Pouring:** In this method amount of the slurry is put on a given size plate which is kept on a level surface. The plate is then tipped back and forth to spread the slurry uniformly over the surface.
2) **Dipping:** This method is developed by Piefer (1962). Plates are prepared by dipping them two at a time back-to-back in chloroform or chloroform-methanol slurries of the absorbent.
3) **Spraying:** A small point sprayer is used for distribution of slurry on a glass plate.

4) **Spreading:** The slurry is placed in an applicator. This is either moved over the stationary plate or it is held stationary and the plate is pushed or pulled.

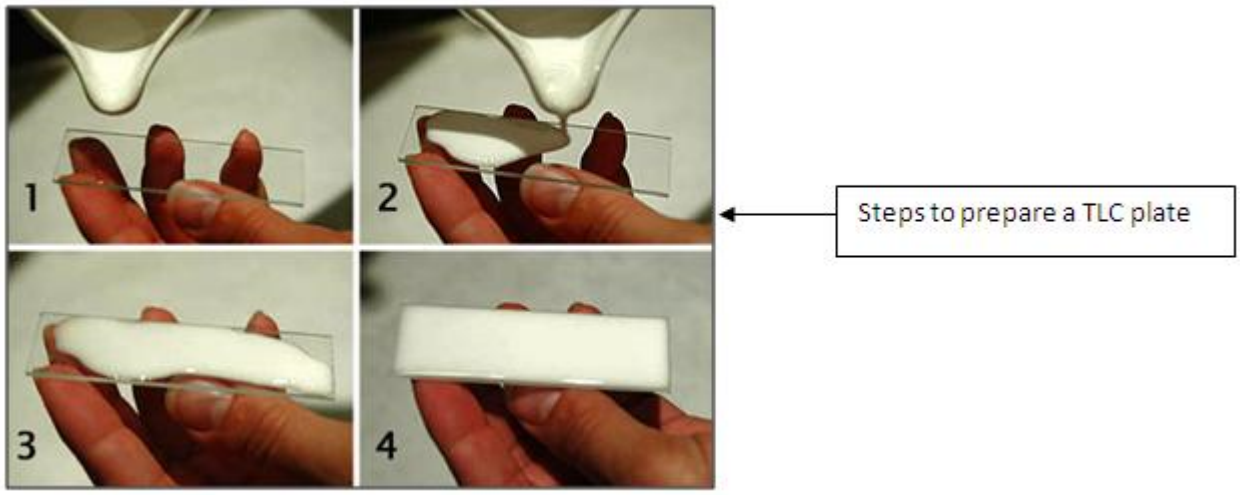

Figure 3.2: Steps for Preparation of TLC Plates

3.6. DEVELOPMENT AND SPOTTING OF TLC PLATES:

Developing a TLC plate requires a developing chamber or vessel. This can be as simple as a wide-mouth jar, but more specialized pieces of glassware to accommodate large plates are available. The chamber should contain enough solvent to just cover the bottom. It should also contain a piece of filter paper, or other absorbent material to saturate the atmosphere with solvent vapors. Finally, it should have a lid or other covering to minimize evaporation (**Figure 3.3** to **Figure 3.5**).

1) Cut the plate to the correct size and using a pencil (never ever use a pen), gently draw a straight line across the plate approximately 1 cm from the bottom. Do not use excessive forces when writing on a TLC plate as this will remove the stationary phase. It is important to use a pencil rather than a pen because inks commonly travel up the plate with the solvent. An example of how black ink separates is shown in the section labeled "examples".
2) Using TLC pipettes, apply spots of analyte to the line. Make sure enough sample is spotted on the plate. This can be done by using the short-wave UV. A purple spot should be seen. If the spot is not visible, more sample needs to be applied to the plate. If a standard of the target compound is available, it is good practice to produce a co-spot by spotting the standard onto a spot of the unknown mixture. This ensures the identity of the target compound.
3) Place the plate into the chamber as evenly as possible and lean it against the side. Never allow the bulk solvent to rise above the line you drew. Allow capillary action to draw the solvent up the plate until it is approximately 1 cm from the end. Never allow the solvent to migrate all the way to the end of the plate.
4) Remove the plate and immediately draw a pencil line across the solvent front.
5) Use a short-wave UV light and circle the components shown with a pencil.

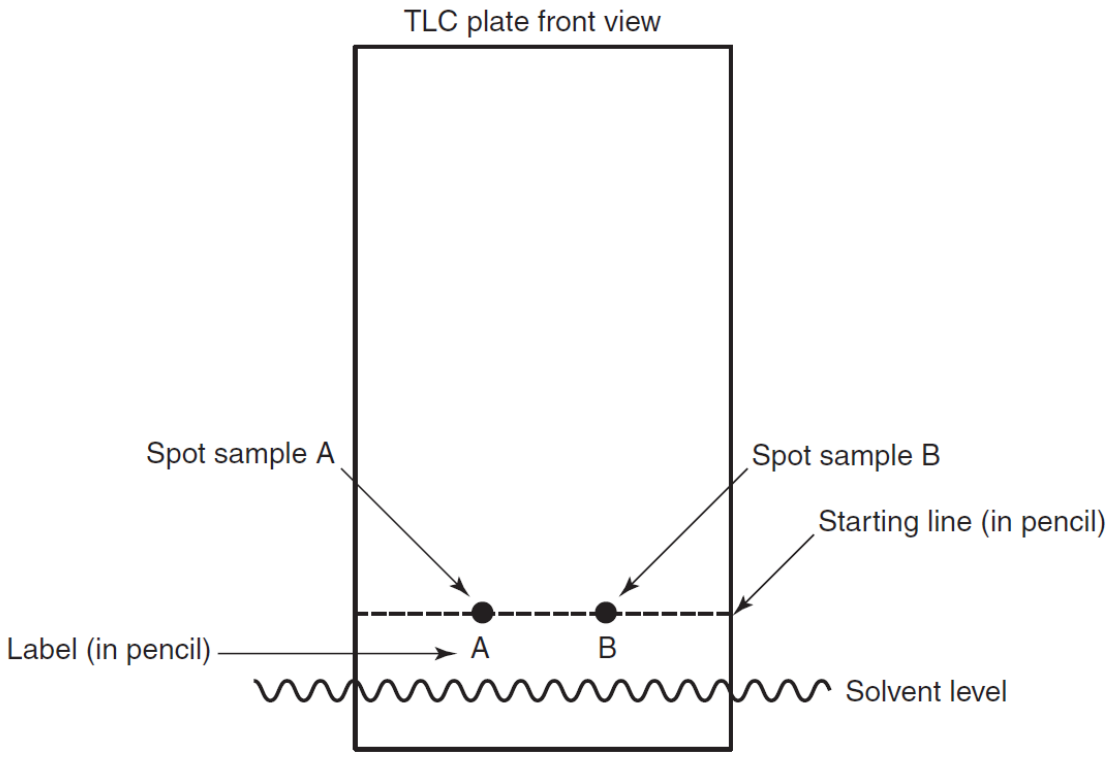

Figure 3.3: Spotting TLC Plate

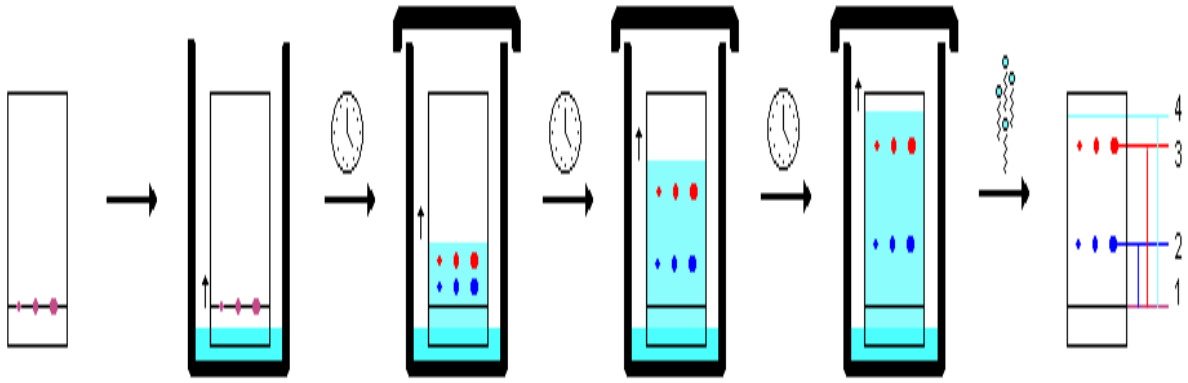

Figure 3.4: The Sequence Involved in Spotting on TLC Plate

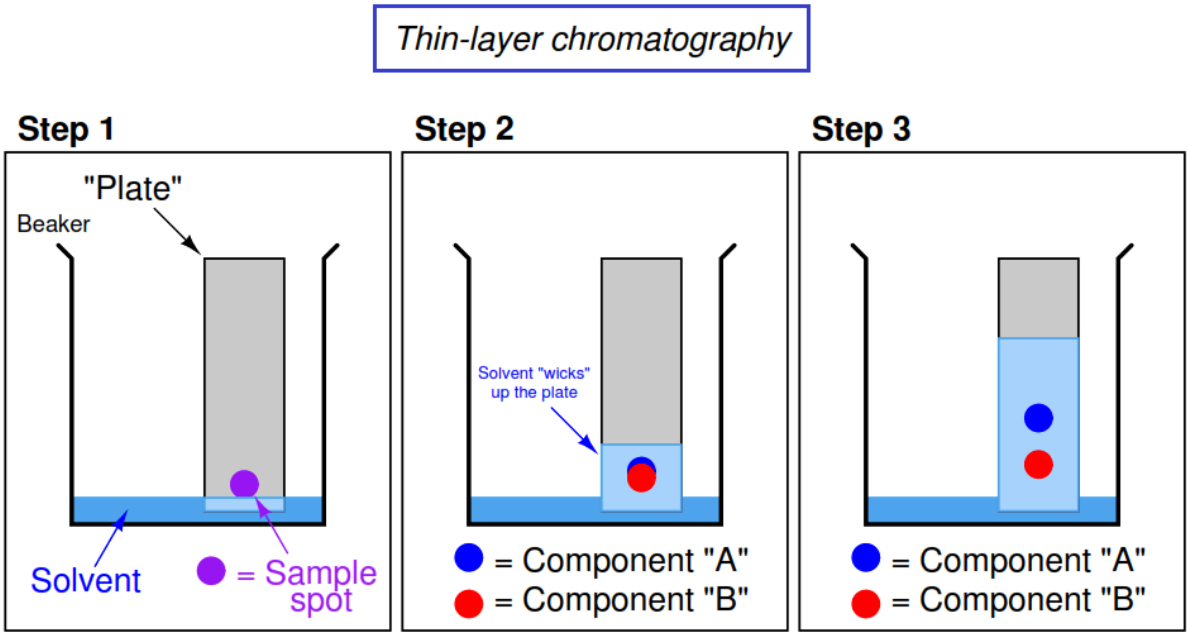

Figure 3.5: The Steps Involved in Spotting on TLC Plate

3.7. THIN LAYER CHROMATOGRAPHY PROCEDURE:

Before starting with the Thin Layer Chromatography Experiment, let us understand the different components required to conduct the procedure along with the phases involved (**Figure 3.6)**.

1) Thin Layer Chromatography Plates – ready-made plates are used which are chemically inert and stable. The stationary phase is applied on its surface in the form of a thin layer. The stationary phase on the plate has a fine particle size and has a uniform thickness.
2) Thin Layer Chromatography Chamber – Chamber is used to develop plates. It is responsible to keep a steady environment inside which will help in developing spots. Also, it prevents the solvent evaporation and keeps the entire process dust-free.
3) Thin Layer Chromatography Mobile phase – Mobile phase is the one that moves and consists of a solvent mixture or a solvent. This phase should be particulate-free. The higher the quality of purity the development of spots is better.
4) Thin Layer Chromatography Filter Paper – It must be placed inside the chamber. It is moistened in the mobile phase.

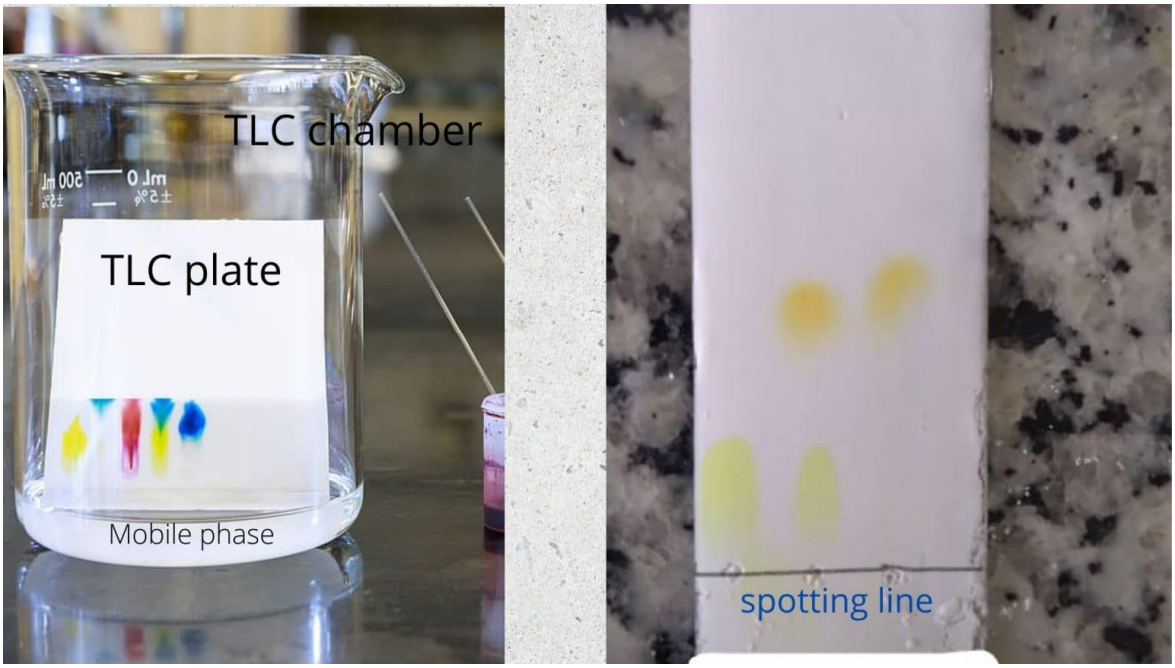

Figure 3.6: Spotting on TLC Plate in TLC Chamber

3.8. THIN LAYER CHROMATOGRAPHY EXPERIMENT:

The stationary phase that is applied to the plate is made to dry and stabilize.

- To apply sample spots, thin marks are made at the bottom of the plate with the help of a pencil.
- Apply sample solutions to the marked spots.
- Pour the mobile phase into the TLC chamber and to maintain equal humidity, place a moistened filter paper in the mobile phase.
- Place the plate in the TLC chamber and close it with a lid. It is kept in such a way that the sample faces the mobile phase.
- Immerse the plate for development. Remember to keep the sample spots well above the level of the mobile phase. Do not immerse it in the solvent.
- Wait till the development of spots. Once the spots are developed, take out the plates and dry them. The sample spots can be observed under a UV light chamber. (**Figure 3.7**)

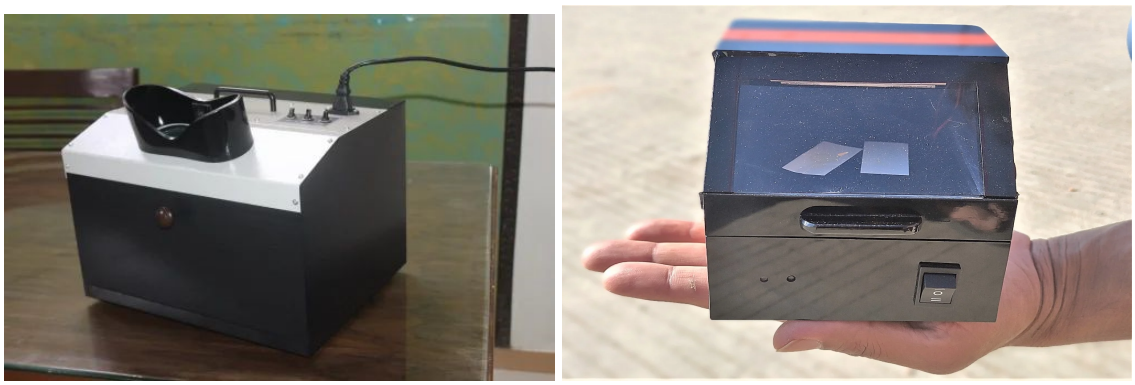

Figure 3.7: UV Light Chamber Cabinet

3.9. VISUALIZATION OF TLC SPOTS:

If fluorescent plates are used, several compounds can be seen by illuminating the plate with short-wave UV. Quenching causes dark spots on the surface of the plate. These dark patches should be circled with a pencil. For compounds which are not UV active, several chemical stains can be used. These can be very general, or they can be specific for a particular molecule or functional group (**Figure 3.8**).

- Iodine is among the most common stains. Plates are placed in a jar containing iodine crystals, or covered in silica gel with iodine dispersed throughout, for approximately one minute. Most organic compounds will be temporarily stained brown.
- Some popular general use stains are Permanganate, ceric ammonium molybdate (CAM), and p-anisaldehyde. These can be kept in jars which plates are dipped into, or in spray bottles. To develop a plate with permanganate, spray or dip the plate and heat it with a heat-gun. Hold the plate face up 10 to 20 cm above the heat gun until the bulk water evaporates. Then move the plate to 5 to 10 cm above the heat gun and heat it until white/yellow/brown spots appear. Overheating will turn the entire plate brown, obscuring the spots. If glass plates are used it is often easier to see spots through the backing because it is harder to overheat. CAM and p-anisaldehyde stained plates are developed similarly. Overheating CAM-stained plates turn everything blue.
- In the case of lipids, the chromatogram may be transferred to a polyvinylidene fluoride membrane and then subjected to further analysis, for example mass spectrometry, a technique known as far-eastern blot.

Once visible, the Rf value, or retardation factor, of each spot can be determined by dividing the distance the product traveled by the distance the solvent front traveled using the initial spotting site as reference. These values depend on the solvent used and the type of TLC plate and are not physical constants.

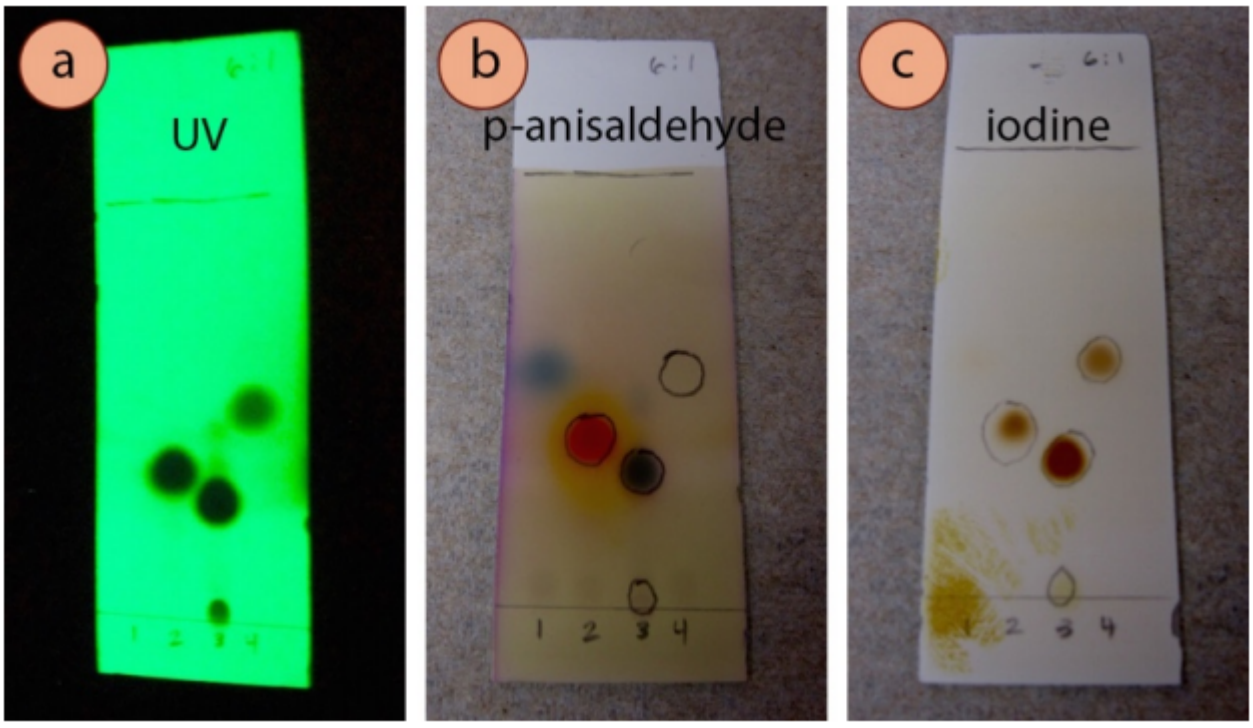

Figure 3.8: Visualizing TLC Plates

3.10. RETENTION FACTOR (R_f):

After a separation is complete, individual compounds appear as spots separated vertically. Each spot has a retention factor (Rf) which is equal to the distance migrated over the total distance covered by the solvent **(Figure 3.9)**.

The formula is

$$Rf = \frac{\text{Distance traveeled by the solute}}{\text{Distance traveeled by the solvent}}$$

The R_f value can be used to identify compounds due to their uniqueness to each compound. When comparing two different compounds under the same conditions, the compound with the larger R_f value is less polar because it does not stick to the stationary phase if the polar compound, which would have a lower R_f value.

The Following Factors Affect the Retardation Factor (Rf):

- Solvent System
- Absorbent (grain size, water content, thickness)
- Amount of Material Spotted
- Temperature

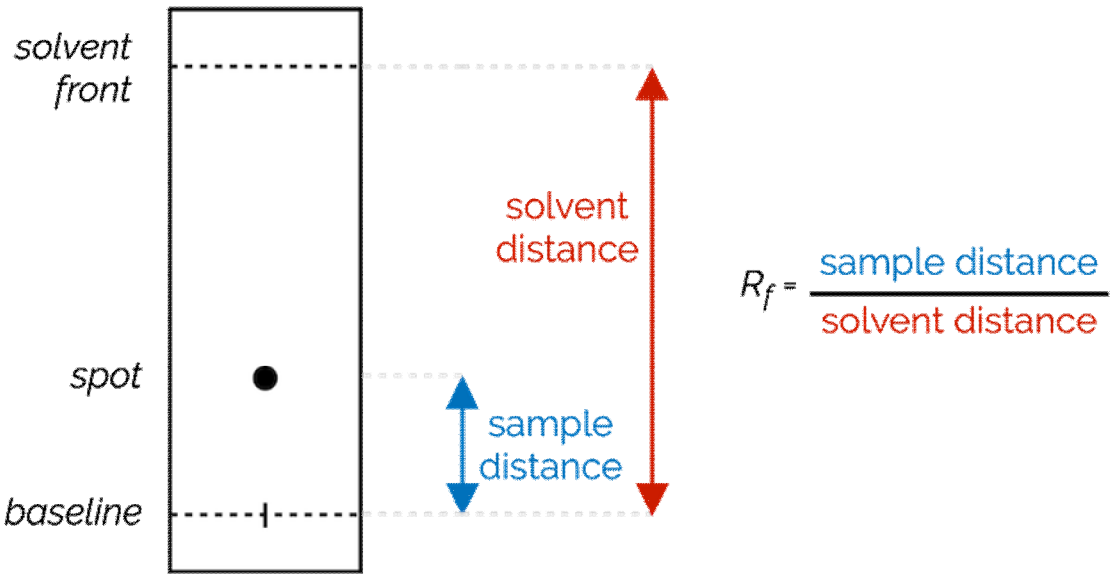

Figure 3.9: Calculation of Rf value using on TLC Plate

R_f values and reproducibility can be affected by several different factors such as layer thickness, moisture on the TLC plate, vessel saturation, temperature, depth of mobile phase, nature of the TLC plate, sample size, and solvent parameters. These effects normally cause an

increase in R_f values. However, in the case of layer thickness, the R_f value would decrease because the mobile phase moves slower up the plate. While R_f can never be greater than 1.

3.11. THIN LAYER CHROMATOGRAPHY APPLICATIONS:

- **Purity of any sample:** TLC can be used to determine the sample's purity. The sample and the standard or authentic sample are directly compared; if any impurities are found, they thus show up as extra spots and are easily detectable.
- **Identification of compounds:** Natural products like volatile oil or essential oil, fixed oil, waxes, alkaloids, glycosides, steroids, etc. can also be purified, isolated, and identified using thin layer chromatography.
- **Analyzing reactions:** Thin layer chromatography can be used to determine whether a reaction is complete or not. Other separation and purification processes, such as distillation and molecular distillation, are also checked using this technique.
- **Biochemical analysis:** TLC is incredibly useful in isolating or separating biochemical metabolites or constituents from its body fluids, such as blood plasma, serum, urine, etc.
- **In chemistry**: TLC methodology is increasingly used in chemistry for the separation and identification of compounds that are closely related to each other. In inorganic chemistry, it is also used to identify cations and anions.
- **In the pharmaceutical industry**: TLC technique has also been adopted by a number of pharmacopeia to identify impurities in pharmacopoeial chemicals.
- The TLC method has been used to qualitatively test several medications, including hypnotics, sedatives, anticonvulsant tranquilizers, antihistamines, analgesics, local anaesthetics, and steroids.
- Separating multicomponent pharmaceutical formulations is therefore one of the most significant uses of TLC.
- Colores, preservatives, sweeteners, and different cosmetic products are separated and identified using the TLC method in the food as well as cosmetic industries.

3.12. THIN LAYER CHROMATOGRAPHY ADVANTAGES:

- This is a very simple method for separating the components.
- Very few types of equipment are used in comparison to other separation methods.
- Because the components elute quickly, they can be separated in a short amount of time.
- All of UV light's constituents are easily observable.
- Some TLC plates lack lengthy stationary phases. In these circumstances, it puts a cap on how long the mixture can be separated. The separation of the mixture into individual components would be finer the longer the plate was.
- The TCL method is used to separate the non-volatile compounds.

- In TLC, only a small sample size is necessary, and it can be measured in microliters.
- A comparison with standard material allows for a preliminary identification.
- The components in the complex mixture of samples can also be easily separated and recovered by scratching the plate.

3.13. THIN LAYER CHROMATOGRAPHY DISADVANTAGES:

- TLC results are difficult to reproduce.
- Despite its simplicity and convenience, one of TLC's drawbacks is that it cannot distinguish between a compound's isomeric and enantiomeric forms. This is because a molecule's chiral pairs share the same physical characteristics.
- It is only possible to add soluble components to the mixtures.
- Only qualitative analysis is feasible; and hence quantitative analysis is not possible.
- Typically, TLC does not happen automatically.
- Because TCL operates in an open system, changes in temperature and humidity can have an impact on the outcomes.

3.14. SUMMARY:

- To know about the Basic Principle in involved in thin layer Chromatography.
- To study about the selection of Stationary Mobile phases.
- To know about Development and Spotting of TLC plates.
- To study about methods of preparing TLC plates.
- To know the importance of R_f value.
- To know about Visualization methods for TLC.
- To study the applications, advantages, disadvantages of TLC.

3.15. SELF ASSESSMENT QUESTIONS:

1) Discuss the methods of preparing TLC plates.
2) Write the selection of mobile phase and stationary phase in TLC.
3) Explain the development of TLC plates.
4) What is the principle involved in TLC and write advantages and disadvantages of TLC.
5) Write about visualization methods in TLC.
6) What is R_f value? Write the factors affecting R_f value.

3.16. REFERENCE BOOKS:

1) Principles of Instrumental Analysis by D.A. Skoog, F.J. Holler and T.A. Nieman, Harcourt College Pub.

2) Separation Techniques by M.N. Sastri, Himalaya Publishing House (HPH), Mumbai.

3) A Hand Book of Instrumental Techniques for Analytical Chemistry- Ed-F.A. Settle, Prearson Edn.

Dr. K. Bala Murali Krishna

LESSON-4

PAPER CHROMATOGRAPHY

4.0. OBJECTIVES:

After studying this lesson, you should be able to:

- To know about the Basic Principle in involved in Paper Chromatography.
- To know about the different types of in Paper Chromatography.
- To study about the selection of Stationary and Mobile phases.
- To know about the ascending and descending types of in Paper Chromatography.
- To learn about Development of chromatograms in Paper chromatography.
- To study about methods of preparing TLC plates.
- To know the importance of R_f value in Paper chromatography.

STRUCTURE:

4.1 Introduction

4.2 Types of Paper Chromatography

4.3 Basic Principle of Paper Chromatography

4.4 Selection of Mobile Phase for Paper Chromatography

4.5 Selection of Stationary Phase and Papers

4.6 Types of Paper Chromatography

4.7 Retardation Factor (R_f) Values

4.8 Development of Chromatograms

4.9 Summary

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4.1. INTRODUCTION:

Paper chromatography (PC) is a type of planar chromatography whereby chromatography procedures are run on a specialized paper.

PC is the simplest and most widely used of the chromatographic techniques because of its applicability to isolation, identification, and quantitative determination of organic and inorganic compounds.

It was first introduced by German scientist Christian Friedrich Schonbein (1865).

Paper chromatography is defined as the technique in which the analysis of an unknown substance is carried out mainly by the flow of solvent on specially designed filter paper. The separation is affected by the differential migration of the mixture of substances. This takes place because of difference in partition coefficients **(Figure 4.1)**.

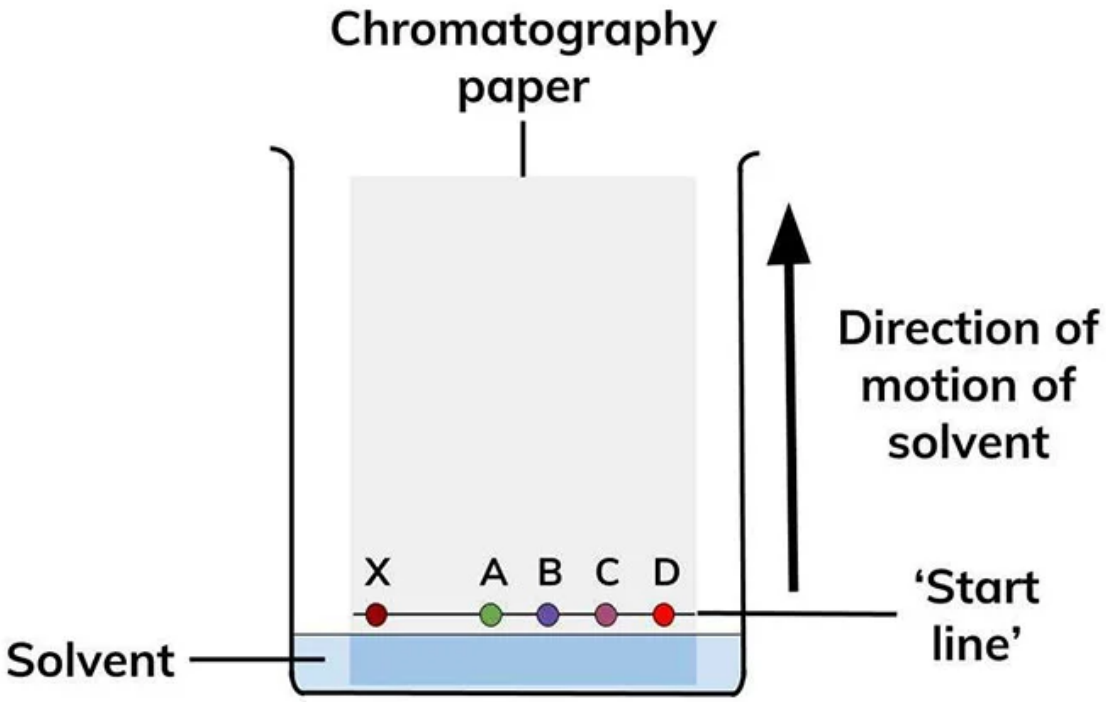

Figure 4.1: Paper Chromatography Diagram

4.2. TYPES OF PAPER CHROMATOGRAPHY:

Paper Adsorption Chromatography:

Paper impregnated with silica or alumina acts as adsorbent (stationary phase) and solvent as mobile phase.

Paper Partition Chromatography:

Moisture / Water present in the pores of cellulose fibers present in filter paper acts as stationary phase & another mobile phase is used as solvent. In general paper chromatography mostly refers to paper partition chromatography.

4.3. BASIC PRINCIPLE OF PAPER CHROMATOGRAPHY:

The principle of separation is mainly partition rather than adsorption. Substances are distributed between a stationary phase and a mobile phase. Cellulose layers in filter paper contain moisture which acts as a stationary phase. Organic solvents/buffers are used as mobile phase. The developing solution travels up the stationary phase carrying the sample with it. Components of the sample will separate readily according to how strongly they adsorb onto the stationary phase versus how readily they dissolve in the mobile phase **(Figure 4.2)**.

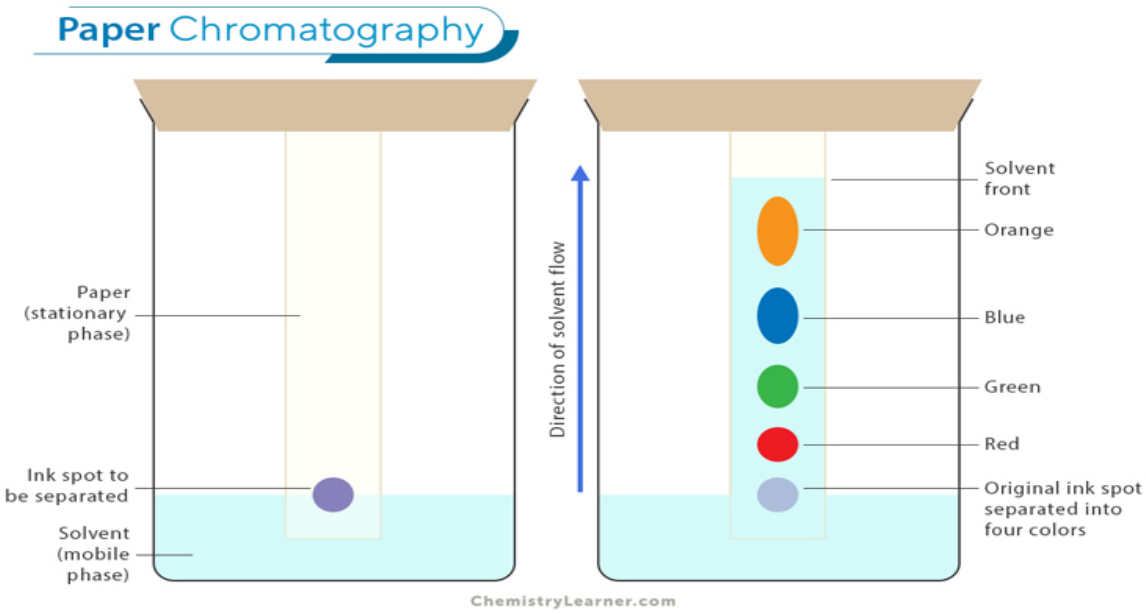

Figure 4.2: Representation of Paper Chromatography

When the movement of mobile phase is in upward direction, the development is called Ascending Development and when it is in down ward direction, the development is known as Descending Development.

4.4. SELECTION OF MOBILE PHASE FOR PAPER CHROMATOGRAPHY:

Pure solvents, buffer solutions, or mixture of solvents can be used.

Examples:

Hydrophilic Mobile Phase:

- Isopropanol: ammonia: water 9:1:2
- Methanol: water 4:1
- N-butanol: glacial acetic acid: water 4:1:5

Hydrophobic Mobile Phases:

- Dimethyl ether: cyclohexane kerosene: 70% isopropanol
- The commonly employed solvents are the polar solvents, but the choice depends on the nature of the substance to be separated.
- If pure solvents do not give satisfactory separation, a mixture of solvents of suitable polarity may be applied.

4.5. SELECTION OF STATIONARY PHASE AND PAPERS:

- Whatman filter papers of different grades like No.1, No.2, No.3, No.4, No.20, No.40, No.42 etc.
- In general the paper contains 98-99% of α-cellulose, 0.3 – 1% β -cellulose.

Other Modified Papers:

- Acid or base washed filter paper
- Glass fiber type paper.
- Hydrophilic Papers – Papers modified with methanol, formamide, glycol, glycerol etc.
- Hydrophobic Papers – Acetylation of OH groups leads to hydrophobic nature, hence can be used for reverse phase chromatography.
- Impregnation of silica, alumna, or ion exchange resins can also be made.

Choice of Filter Papers:

- Various types of Whatman chromatographic papers are available. The choice of paper depends
- upon the type of separation. Generally coarser and faster papers, Whatman 31ET, are used when the
- substances to be separated have wide - apart RF values.
- Whatman filter paper commonly used for chromatographic purpose has been found to contain
- 99% α-cellulose and the rest is mineral content.

Suitable Solvent Systems for Paper Chromatography:

Stationary Phase	***Mobile Phase***
Water	Phenol saturated with water
Water	n-butanol - acetic acid - water (4: 1: 5)
Formamide	CHCl3
Formamide	Benzene
Kerosene	70% Iso propanol
Phenoxy Ethanol	Heptane.

4.6. TYPES OF PAPER CHROMATOGRAPHY:

There are different types of chromatographic techniques are available for paper chromatography.

(a) Descending Chromatography:

When the development of the paper is done by allowing the solvent to travel down the paper, it is known as descending technique.

Descending chromatography, also known as column chromatography, is a type of chromatography that involves the separation of components of a mixture based on their affinity to a stationary phase.

In this technique, the mixture is loaded onto a column containing a stationary phase, and a solvent is passed through the column. The components of the mixture are separated based on their interaction with the stationary phase, with the more strongly adsorbed components eluting later than the weakly adsorbed components (**Figure 4.3**).

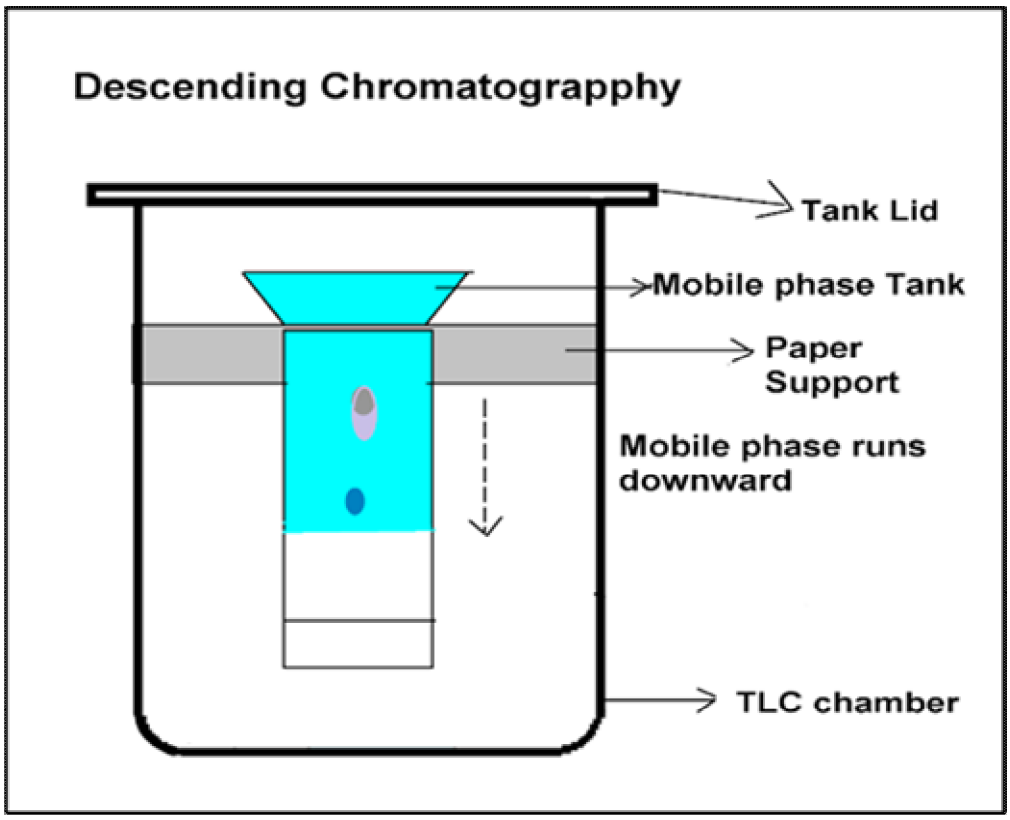

Figure 4.3: **Descending Paper Chromatography**

Advantages of Descending Chromatography:

One of the advantages of descending chromatography is that it provides higher resolution compared to ascending chromatography. It is also more versatile and can be used for a wide range of applications, including preparative chromatography for the purification of compounds.

(b) Ascending Chromatography:

When the development of the paper is done by allowing the solvent to travel up the paper, it is known as ascending technique.

Ascending chromatography is a type of paper chromatography that involves the separation of components of a mixture based on their ability to migrate through a stationary phase.

In this technique, a small spot of the sample is applied to the bottom of a chromatography paper, and the paper is then placed in a solvent. As the solvent rises up the paper, it carries the components of the mixture with it, separating them based on their solubility and polarity (**Figure 4.4**).

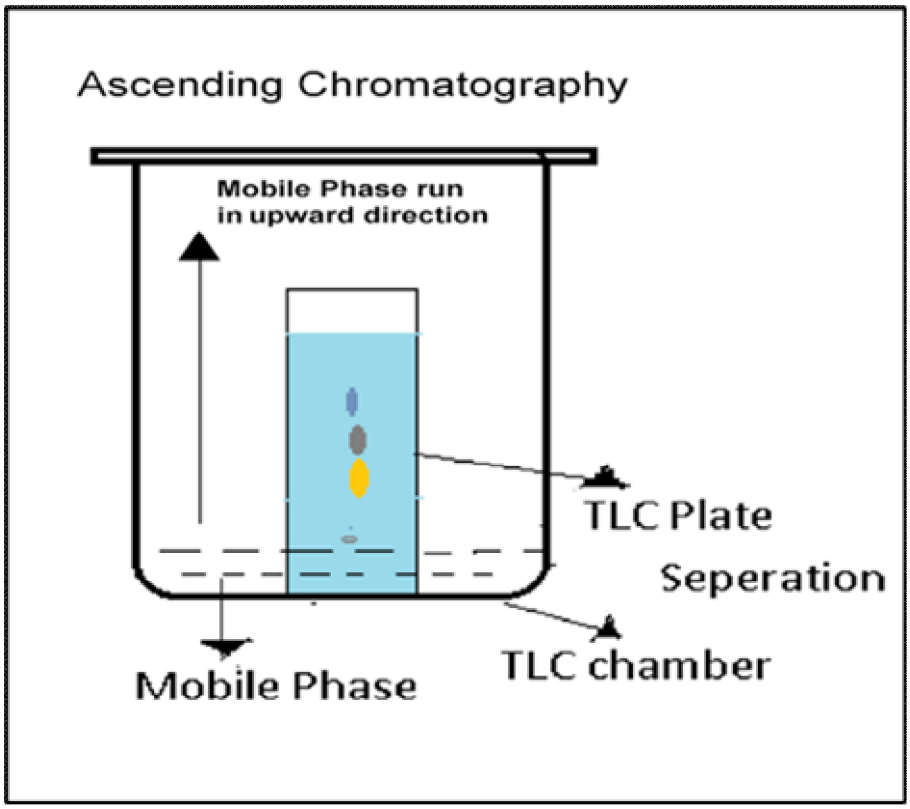

Figure 4.4: Ascending Chromatography

Advantages of Ascending Chromatography:

One of the advantages of ascending chromatography is that it is simple and inexpensive, requiring minimal equipment and materials. However, it can be time-consuming, and the resolution is often lower compared to other chromatography techniques.

(c) Ascending – Descending Chromatography:

The upper part of the ascending chromatography can be folded over a glass rod allowing the ascending development to change over into the descending after crossing the glass rod.

(d) Radial Paper Chromatography:

This is also known as circular paper chromatography. This makes use of radial development. A circular filter paper is generally employed. The various materials to be analyzed are placed at its center. After drying the spots, the paper is fixed horizontally on the petri dish possessing the solvent so that the tongue or wick of the paper dips into the solvent.

Cover the paper by means of petri dish covers. The solvent rises through the tongue or the wick, the components gets separated in the form of concentric zones.

4.7. RETARDATION FACTOR (R_f) VALUES:

Some compounds in a mixture travel almost as far as the solvent does; some stay much closer to the baseline. The distance travelled relative to the solvent is a constant for a particular compound as long as other parameters such as the type of paper and the exact composition of the solvent are constant. The distance travelled relative to the solvent is called the Rf value (**Figure 4.5**).

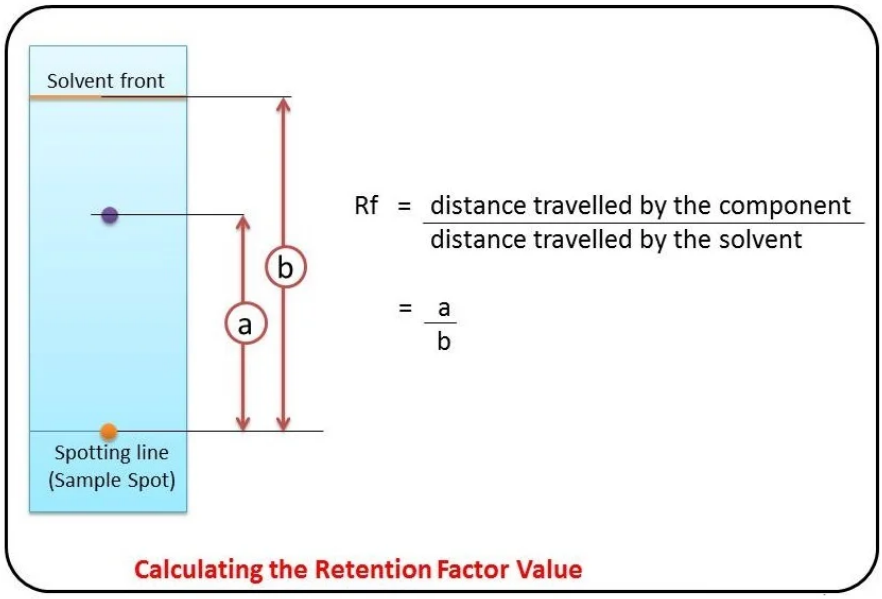

Figure 4.5: Calculation of Rf Values

Thus, in order to obtain a measure of the extent of movement of a component in a paper chromatography experiment, “Rf value” is calculated for each separated component in the developed chromatogram. A Rf value is a number that is defined as the distance travelled by the component from the application point.

In many cases it has been observed that the solvent front is run off the end of the paper. It is the ratio of distance travelled by the sample and the distance travelled by the standard. Rx value is always closer to 1.

Factors Affecting Rf Values:

- The temperature
- The purity of the solvents used
- The quality of the paper, adsorbents & impurities present in the adsorbents ϖ * Chamber saturation techniques, method of drying & development ϖ The distance travelled by the solute & solvent.
- Chemical reaction between the substances being partitioned.
- pH of the solution

4.8. DEVELOPMENT OF CHROMATOGRAMS:

The development of a chromatogram is a crucial part of any of the chromatographic techniques. Clear separation of the employed for the development of a chromatogram. The advent of chromatographic development can be Arne Tiselius who categorized chromatographic development methods into three basic types which are:

i) Frontal analysis

ii) Displacement development

iii) Elution analysis

i) Frontal Analysis:

In the frontal analysis procedure of chromatographic development, the mixture to be separated is feed continuously into the column under suitable conditions that selectively favour the binding of all the components of the mixture excluding just one component. The component which is least retained in the stationary phase can be obtained in pure form at the column outlet. In this process of chromatographic development, no additional mobile phase is used. **Figure 4.6** describes the frontal analysis method of chromatographic development.

This frontal analysis method is usually applied in the purification of biopolymers where the desired component to be separated has a much lower affinity for the stationary phase as compared to the rest of the feed component.

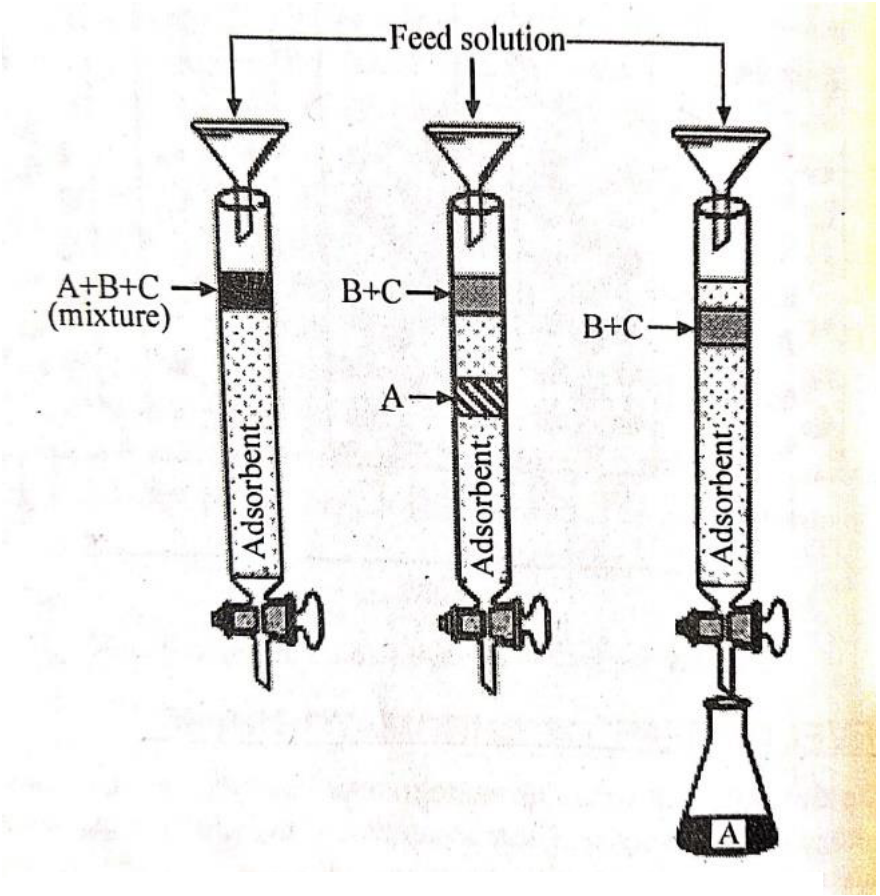

Figure 4.6: Graphical Representation if Frontal Analysis Method

ii) Displacement Development:

This chromatographic development procedure is employed as a preparative technique mainly in the pharmaceutical industry to purify or separate active ingredients of a complex mixture from impurities.

In this chromatographic development technique, a molecule called displacer is used to displace other analyte molecules that were already adsorbed into the stationary phase. In this development procedure, the mixture of sample to be separated is first loaded into the chromatographic bed containing the stationary phase. Then the mobile phase containing a suitable displacement reagent is allowed to pass through the chromatographic bed. The selection of the displacement reagent should be made in such a way that it has a very strong binding affinity towards the stationary phase as compared to all the individual components of the analyte. The displacer will displace the different components of the analytes at different retention times depending on their affinity of binding towards the stationary phase. The analyte component with least binding affinity will be displaced more easily than the others and will be eluted first.

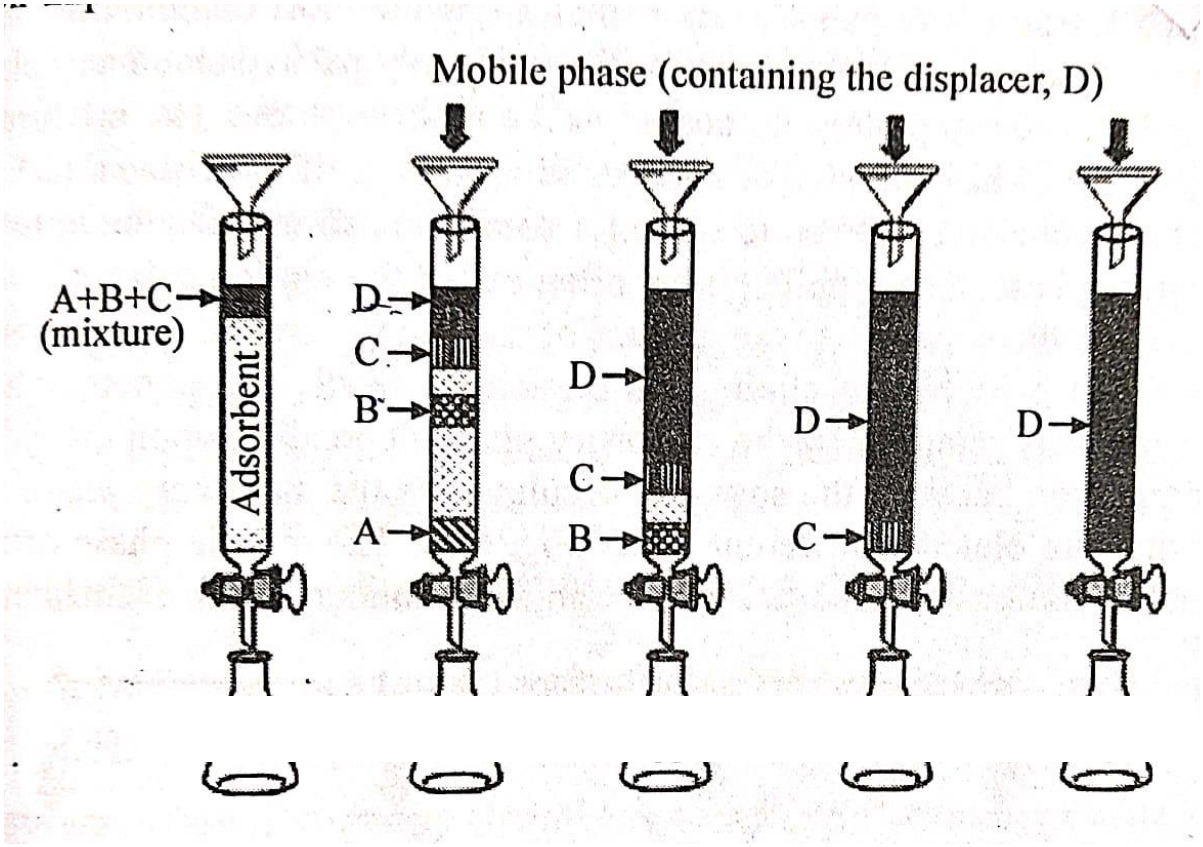

Figure 4.7: Graphical Representation of Displacement Analysis Method

Thus, in the displacement chromatographic development technique, consecutive zones of separated components have been formed throughout the chromatographic bed and can be eluted in accordance with their relative affinity for the stationary phase. After displacing all the analyte molecules, the chromatographic bed should be regenerated (i.e. complete removal of all the displacer molecules) (**Figure 4.7**).

iii) Elution Analysis:

Elution analysis is the most common mode of chromatographic development where the uniformly packed stationary phase in a column is completely immersed in the mobile phase. The mixture of components to be separated is introduced at the top of the column to form a uniform zone and allowed the settle for some time so that it adheres to the stationary phase. The mobile phase, often called the eluting solvent is the poured into the column and the process of passing through the column is known as elution. As elution proceeds, component solutes are selectively retarded by the stationary phase depending upon the extent of interaction between the solute molecules with the stationary phase and thus they are eluted at different times (**Figure 4.8**).

The mobile phase carries the solute molecules down the column in a continuous series of transfers as the solute molecules partitioned themselves between the two phases.

In other words, solutes having strong interaction with the stationary phase will be retained mostly in that phase and those having poor interaction with the stationary phase will spend mostly in the mobile phase. Thus, due to these differences, the rate of migration of different components are different that causes them to separate into bands or zones along the length of the column. Pouring enough of the mobile phase through the column, the individual band of each component can be isolated.

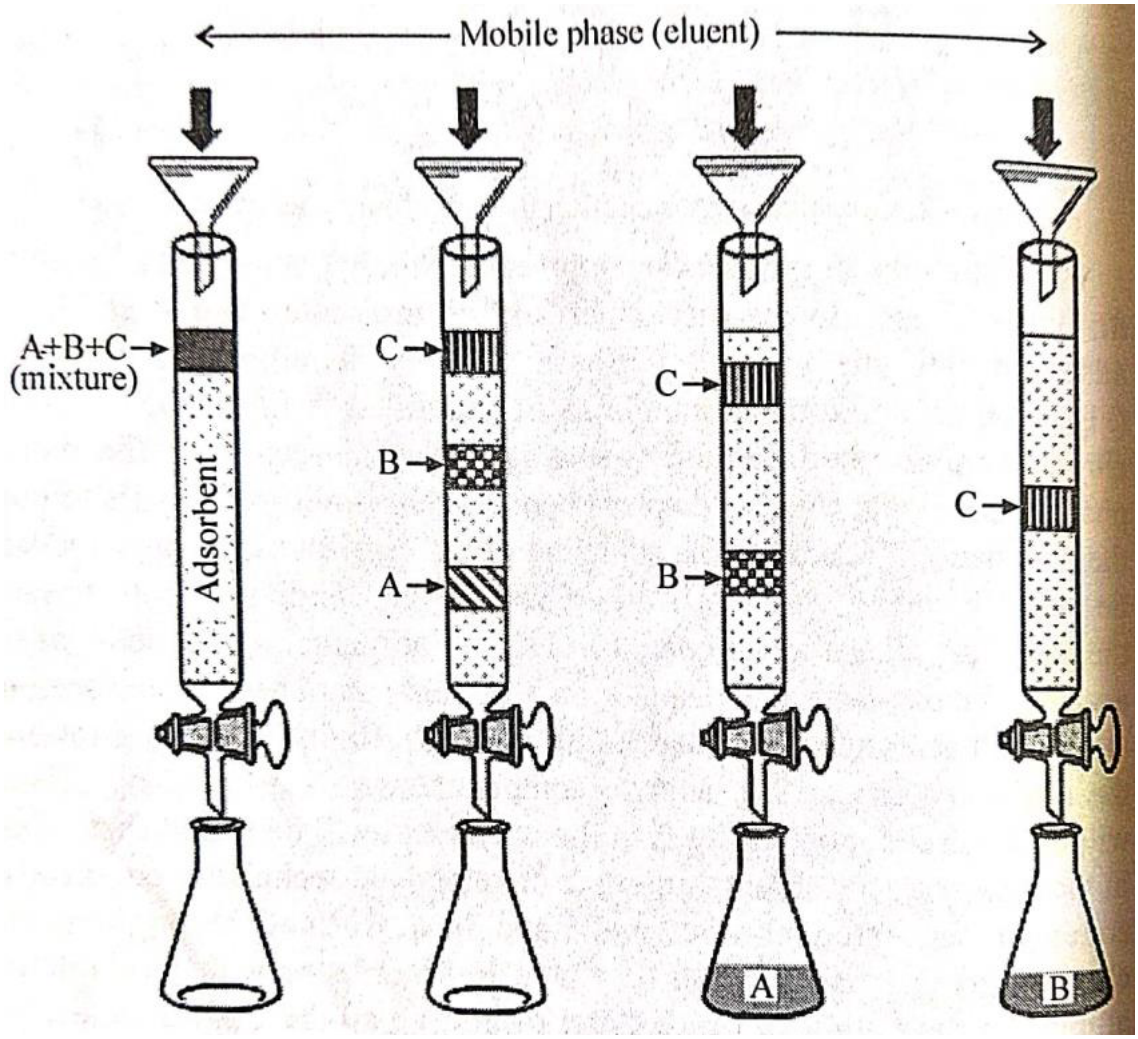

Figure 4.8: Graphical Representation of Elution Method

Isocratic and Gradient Elution:

Elution may be of two types isocratic and gradient. In the isocratic method, the mobile phase used is of the same composition during the separation process. But in the gradient method, the polarity or strength of the mobile phase is changed time to time to make analysis shorter or to get a better resolution of the components.

4.9. SUMMARY:

- To know about the Basic Principle in involved in Paper Chromatography.
- To know about the different types of in Paper Chromatography.
- To study about the selection of Stationary and Mobile phases.
- To know about the ascending and descending types of in Paper Chromatography.
- To learn about Development of chromatograms in Paper chromatography.
- To study about methods of preparing TLC plates.
- To know the importance of R_f value in Paper chromatography.

4.10. SELF ASSESSMENT QUESTIONS:

1) Discuss the ascending and descending techniques in Paper chromatography.
2) Write the selection of mobile phase and stationary phase in Paper chromatography.
3) Explain the development of chromatograms in Paper chromatography.
4) What is the principle involved in PC and write advantages and disadvantages of PC.
5) What is R_f value? Write the factors affecting R_f value.

4.11. REFERENCE BOOKS:

1) Principles of Instrumental Analysis by D.A. Skoog, F.J. Holler and T.A. Nieman, Harcourt College Pub.
2) Separation Techniques by M.N. Sastri, Himalaya Publishing House (HPH), Mumbai.
3) A Hand Book of Instrumental Techniques for Analytical Chemistry- Ed-F.A. Settle, Prearson Edn.

Dr. K. Bala Murali Krishna

LESSON-5

ONE AND TWO DIMENSIONAL PAPER CHROMATOGRAPHY

5.0. OBJECTIVES:

After studying this lesson, you should be able to:

- To know about in one dimensional or two-dimensional Paper Chromatography.
- To know about the different visualization methods for Paper Chromatography.
- To learn about Identification of amino acids using in Paper chromatography.
- To learn about Identification of sugars using in Paper chromatography.
- To study about applications, advantages, and disadvantages of Paper chromatography.

STRUCTURE:

5.1 One Dimensional Paper Chromatography

5.2 Two-Dimensional Paper Chromatography

5.3 Detecting or Visualization Methods

5.4 Application of Paper Chromatography in the Identification of Amino Acids.

5.5 Application of Paper Chromatography in the Identification of Sugars.

5.6 Applications of Paper Chromatography

5.7 Advantages of Paper Chromatography

5.8 Disadvantages of Paper Chromatography

5.9 Limitations of Paper Chromatography

5.10 Summary

5.11 Self-Assessment Questions

5.12 Reference Books

Paper chromatography may be one dimensional or two dimensional depending upon the type of complexity involved in the analysis.

5.1. ONE DIMENSIONAL PAPER CHROMATOGRAPHY:

A strip of filter paper usually 15 to 30cm in length and one to several centimetres in width is first laid flat. A minute drop of the sample solution is placed in the centre an inch from one end of the paper and its position is marked by a pencil. The original solvent is allowed to evaporate to make the spot of the sample dry. The portion of the paper nearest the sample spot is then brought in contact with a suitable solvent, called developer. Both paper and developer are sealed in container. After some time (~ 6hrs) the liquid rises up the strip by

capillary action, carrying the constituents of the sample along with it at various speeds, according to their partition coefficients. After the liquid has traversed the length of the strip, the paper is removed and dried. The position of the solvent front is marked with a pencil at the two edges of the paper and the so-called chromatogram is then dried by keeping in an oven or over a hot plate for a few minutes. Drying is best carried out by means of a fan or hairdryer.

The finished dried paper thus obtained is called paper chromatogram. If the substances are coloured no difficulty arises, but in case of colourless substance a few physical and chemical methods are employed for locating the spots (**Figure 5.1**).

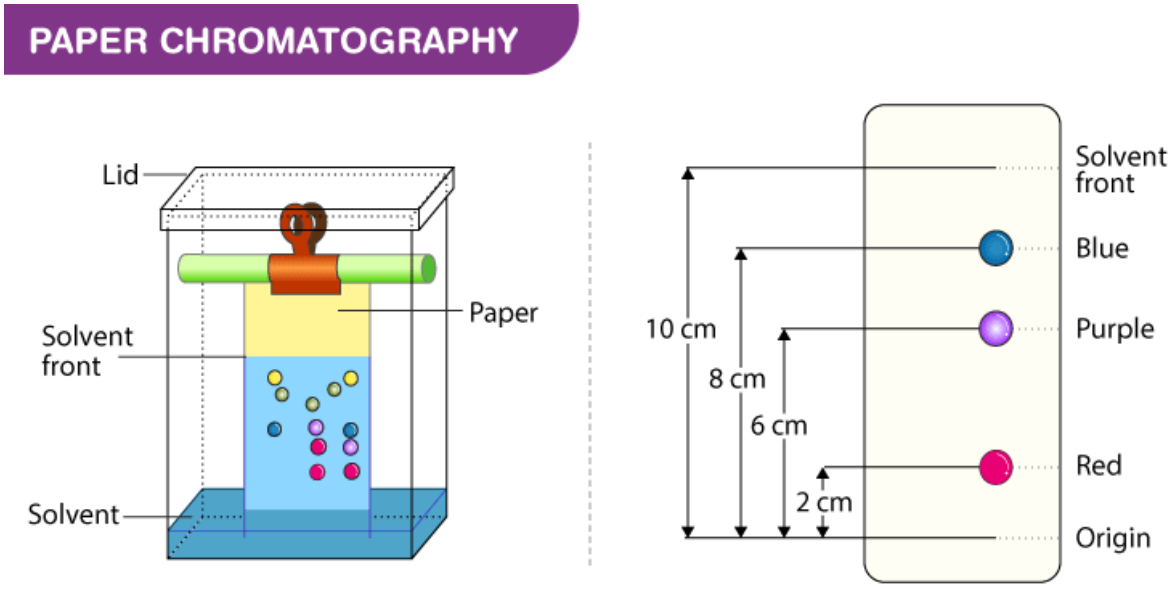

Figure 5.1: One Dimensional Paper Chromatography

5.2. TWO-DIMENSIONAL PAPER CHROMATOGRAPHY:

In this method the sample is placed on one corner of a square sheet of paper. Development along axis is then performed as described above. After the evaporation of the solvent the paper is rotated through 90^{o} so that the edge having the series of spots is now at bottom and is again developed but with different solvent (**Figure 5.2**).

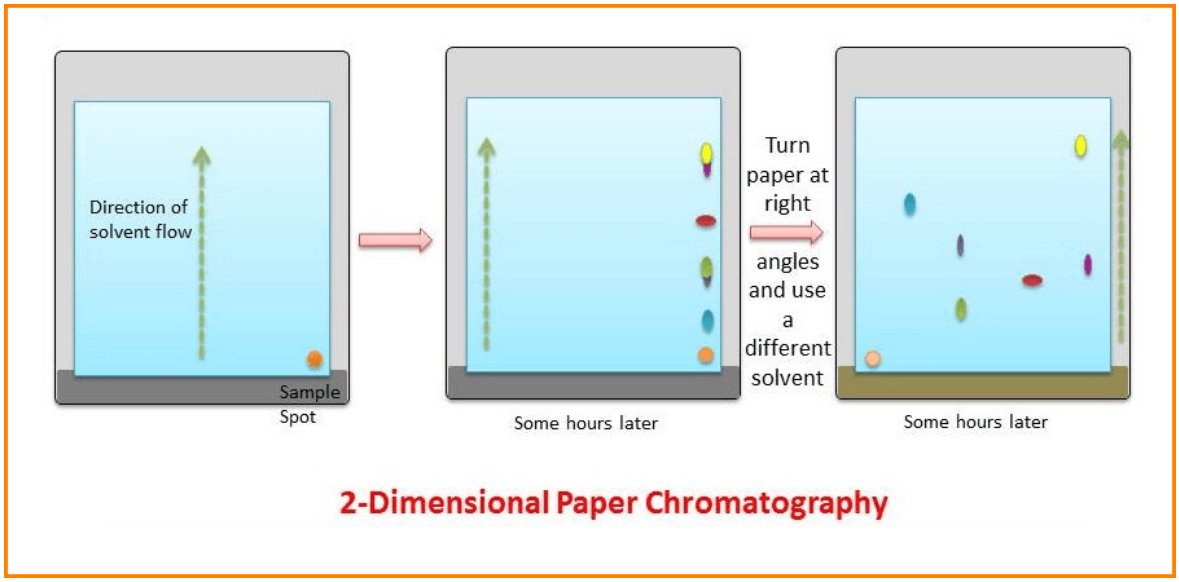

Figure 5.2: Two dimensional Paper Chromatography

Two-dimensional chromatography is especially suitable for those substances which cannot be separated by one-dimensional chromatography. This happens when the RF values are very close and nearly the same.

5.3. DETECTING OR VISUALIZATION METHODS:

Coloured spots are easily observed on developed chromatograms. However, different approaches need to be adopted when colourless components are to be observed. It is convenient to classify such methods as specific or non-specific.

Non-specific methods (Physical):

Iodine chamber: The developed plate is suspended in a closed jar containing a few crystals of iodine for about a minute. In presence of iodine vapour most organic compounds appear as brown spots.

UV viewing cabinet: Majority of colourless compounds can be viewed under illumination with UV light in a UV viewing cabinet. Commonly the cabinets are equipped with a long wavelength (366 nm) and short wavelength (254 nm) light sources.

***Specific methods (Chemical methods)*:**

- Ferric chloride
- Ninhydrin in acetone
- Dragendroff's reagents
- 3,5-dinitro benzoic acid
- Molisch reagent
- Phenolic comp. & tannins

- Amino acids
- Alkaloids
- Cardiac glycosides
- Carbohydrates

5.4. APPLICATION OF PAPER CHROMATOGRAPHY IN THE IDENTIFICATION OF AMINO ACIDS.:

Aim:

To separate and identify the components of a mixture of amino acids using paper chromatography.

Theory>

Chromatography is a powerful analytical technique used to separate the components of a mixture based on their differential affinities towards a stationary phase and a mobile phase. In paper chromatography, the stationary phase is a sheet of cellulose-based filter paper, and the mobile phase is a suitable solvent or solvent mixture. When a mixture is spotted onto the paper and developed in the solvent, its components travel at different rates depending on their solubility and interaction with the stationary phase. This technique is especially useful in the separation of biomolecules such as amino acids, sugars, and plant pigments.

Principle:

Paper chromatography works on the principle of partition chromatography, where the components of the mixture partition between two phases: the stationary phase (water molecules bound to cellulose in the paper) and the mobile phase (organic solvent). Components with higher affinity for the mobile phase travel faster and farther on the paper, whereas those with higher affinity for the stationary phase travel slower. The separated components can be visualized using specific reagents such as ninhydrin for amino acids. The Rf value (Retention factor) of each component can be calculated to help in its identification.

Requirements:

- Chromatography paper (Whatman No. 1 filter paper)
- Amino acid mixture (e.g., glycine, alanine, serine)
- Individual amino acid standards (optional)
- Capillary tubes or micropipette
- Chromatography jar or glass beaker with lid
- Solvent system (e.g., n-butanol: acetic acid: water in 4:1:5 ratio)
- Pencil and ruler
- Ninhydrin solution (0.1% in acetone or ethanol)
- Spray bottle or cotton swab

- Hot air oven
- Forceps and gloves

Procedure:

1) Cut a strip of chromatography paper approximately 20 cm long and 5 cm wide.
2) Using a pencil (not pen), draw a horizontal line 2 cm from the bottom edge. This is the baseline.
3) Mark 3-4 points equally spaced along the baseline to spot the samples (e.g., sample mixture and standard amino acids).
4) Using a capillary tube or micropipette, apply small spots of the amino acid samples on the marked positions. Allow each spot to dry before reapplying to concentrate the sample.
5) Prepare the chromatography jar by adding about 1 cm depth of the solvent system and cover the jar to allow solvent vapor equilibration.
6) Once the sample spots are dry, suspend the chromatography paper vertically in the jar so that the bottom edge is immersed in the solvent but the spots remain above the solvent level.
7) Close the jar and allow the solvent to rise up the paper undisturbed for about 45–60 minutes or until it is about 2–3 cm from the top.
8) Remove the paper carefully, mark the solvent front with a pencil immediately, and allow the paper to dry.
9) Spray the paper evenly with ninhydrin solution in a fume hood or use a cotton swab to apply it over the chromatogram.
10) Dry the paper using a hot air oven (or hair dryer) at 60-70 °C for 5-10 minutes to visualize the amino acid spots, which appear as purple or blue-violet stains.
11) Measure the distance travelled by each spot and the solvent front from the baseline using a ruler.
12) Calculate the Rf values using the formula:

$$\text{Rf} = \frac{\text{Distance traveeled by the solute}}{\text{Distance traveeled by the solvent}}$$

Observation:

Spot No.	Sample Name	Distance travelled by spot (cm)	Distance travelled by solvent front	R_f value
1	Amino acid A	X_1	Y	X_1/Y
2	Amino acid B	X_2	Y	X_2/Y
3	Amino acid mixture	X_3, X_4	Y	X_3/Y X_4/Y

Note: Rf values can be compared with standard values for identification.

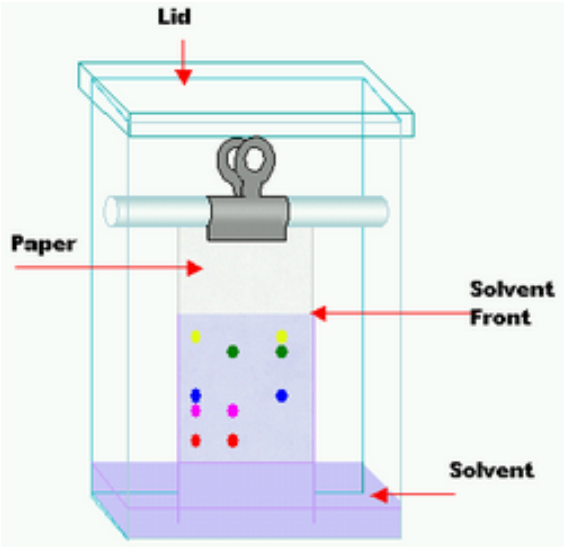

Figure 5.3: Paper Chromatography Chamber

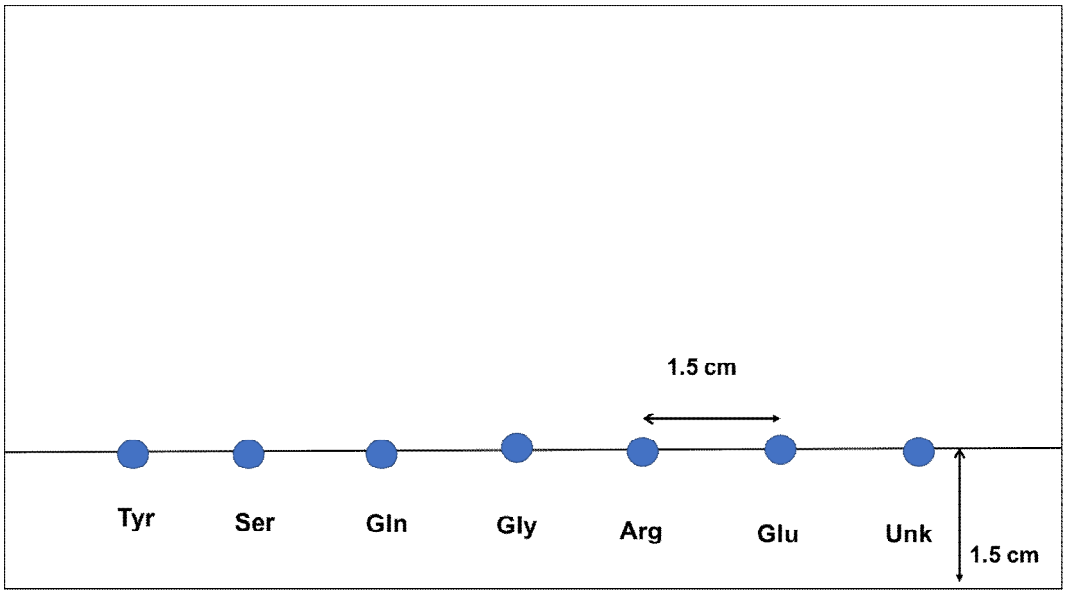

Figure 5.4: Prepared Chromatography Paper with Amino Acids

Result:

The paper chromatogram showed distinct spots for different amino acids present in the mixture _____.

The calculated Rf values matched with those of standard amino acids, confirming their identity_____.

Conclusion:

Paper chromatography successfully separated and identified the amino acids in the mixture based on their differential solubilities and interactions with the mobile and stationary phases.

5.5. APPLICATION OF PAPER CHROMATOGRAPHY IN THE IDENTIFICATION OF SUGARS:

Aim:

To separate and identify the components of sugars using paper chromatography.

Principle:

Distribution of solute (sugar) between the stationary and mobile phases, that is the partition process is the major factor in the PC separation of sugars. Their partition coefficients are substantially in favour of the aqueous phase. Therefore, with non-aqueous developers, sugars appear on the paper choromatogram with low Rf values, whereas with developer containing larger aqueous ratio, the Rf values of sugars are much higher. This is because a sugar molecule containing larger number of hydroxyl groups which is readily soluble in water and makes the partition coefficient in favour of the aqueous phase. Further, the Rf values of sugars are affected by their structural formulae, their molecular mass, the number of -OH ' groups, and presence of other kinds of groups such as aldehydes. or ketones etc.

Requirements:

Apparatus: Chemical Boiling tubes, Measuring cylinder (100 cm), Spotting Capillaries, Spraying-bottle, Whatman No.1 filter paper sheets', Detector.

Chemicals: 1-Butanol, Sugars, Acetic acid.

Solution Provided:

1) **Unknown sugar sample solution:** It can be prepared by dissolving any one or two sugars in water.
2) **Detector:** Any one of the following detectors may be prepared.

Detector-1: Ammoniacal silver nitrate: Take 5 cm^3 of saturated aqueous solution of silver nitrate add 50 cm^3 of acetone, finally add ammonia solution to make the solution clear and basic in nature.

Detector-2: Aniline hydrogen phthalate: Dissolve 1 cm^3 of aniline and 1.66 g of phthalic acid in 100 cm^3 of 1-butanol saturated with water.

Detector-3: p-Anisidine hydrochloride in 100 cm^3 of 1-butanol.

Detector-4: (For non-reducing sugars): Prepare a solution by mixing 0.25~ ' sodium borate + phenol red + methanol in (1:2:7) proportion.

Developer: In a separatory funnel take 1-butanol + acetic acid + water in the proportion (4:1:5) and shake gently. Allow the layers to settle. Remove the lower aqueous layer and take the upper organic phase (layer) as the developer for sugars.

Procedure:

Proceed according to the following steps.

1. Preparation of Solution:

i) **Sample solutions**: Prepare the aqueous solution of any three of the following by dissolving 0.2-0.5 g of each sugar in 5 cm3 of water in a small test tube. The sugars are: D-gulcose, D-fructose, D-xylose, L-rhamnose, D-galactose, Lactose, maltose, sucrose, D-mannose.

ii) **Preparation of mixture solution of sugars:** Add few drops of each sample sugar solution in a dry test tube.

For analysis of sugars from plant or fruit juices, first the material is stored, then the sugars are extracted by grinding the materials in presence of a suitable solvent and finally the non- carbohydrate-material is removed from the extract. The extract can be applied directly or after concentration.

The extract from the fruits contains many other substances along with sugars, for example proteins and organic acids are frequently present in reasonable concentrations to affect the quality of chromatogram.

During the storage of biological material there is always danger of changes in the composition by fermentation of sugars due to the presence of micro-organisms (enzymes).

1) Unknown Sugar solution: It can be prepared by dissolving any one or two sugar in water.
2) Cut the chromatographic paper strips of the required size.
3) On each strip draw a line with pencil at about 1 cm from one end and put a mark at the centre of the line. The sample is to be applied at this mark. Write the name of a particular sugar on the upper side of the paper with pencil.
4) Apply the respective sugar solution to the point of application separately on the marked strips. Use a fresh capillary for each solution.
5) Apply the mixture solution and the unknown solutions separately on other strips.
6) Dry the spots by allowing the solvent to evaporate.
7) Take the clean and dry boiling tubes and place 10-15 mL of the developer in each of these tube.
8) Suspend the spotted and dried paper strips in the respective boiling tubes containing distilled water with upper end pinned to the cork and the lower end touching the developer. Care should be taken to see that this is done gently and the strip is vertical. The spot should always be above the developer level.
9) Allow the developer to rise along the paper and wait till the developer (solvent front) reaches near the upper end of the paper.
10) Remove the paper strip from the boiling tube and mark the solvent front with the help of a pencil.
11) Dry the strip until the acetic acid odour from the strip is no more present. 12. Treat the strip with a detector by a spraying bottle.

12) Heat the strip at 105 ^{o}C in an oven until the coloured zones of sugars are seen.

13) Encircle the coloured zones and mark the centre of each zone.

14) Calculate the Rf values and compare the Rf values of individual sugars with that of their Rf values in mixtures to identify the sugars present in the mixture/sample solution.

Observation and Calculations:

Observe the colour of the spots of various sugars. The colour depends on the detector used. Measure the distance travelled by the centre of the solute zone (ds) and the distance travelled by the solvent front (dm) on the paper choromatogram.

Calculate the Rf values of each sugar by the relation R_f = ds/dm.

Record your data in the following way:

Observation Table:

Paper Chromatography Separation of Sugars of I-Butanol, Acetic Acid end Water (4:1:5)

Sugar	**ds**	**dm**	**Rt = ds/dm Remark**
Lactose			
D-Glucose			
D-Fructose			
Mixture			
Unknown			
1			Rf resemble with ……..
2			Rf resemble with ……..

Results and Discussion:

Sugars present in the unknown sample are:

1. _____

2. _____

5.6. APPLICATIONS OF PAPER CHROMATOGRAPHY:

Paper chromatography has many applications in various fields, some of which are discussed below.

Separation of Amino Acids:

Paper chromatography is commonly used to separate amino acids. Amino acids can be identified by their characteristic Rf values, which are the distances travelled by the amino acid divided by the distance travelled by the solvent.

Forensic Analysis:

Paper chromatography is used in forensic analysis to identify drugs, poisons, and other substances. The characteristic spots on the chromatogram can be used to identify the substance in question.

Food Analysis:

Paper chromatography is used in the food industry to analyze food additives and identify any contaminants present in food. This technique is also used to identify the different pigments present in foods such as fruits and vegetables.

Environmental Analysis:

Paper chromatography is used to analyze environmental samples such as soil and water to identify pollutants and other contaminants.

Pharmaceutical Analysis:

Paper chromatography is used in the pharmaceutical industry to analyze and identify different compounds and their impurities. This is particularly useful in drug development and quality control.

Chemical Education:

Paper chromatography is a common experiment in high school and college chemistry courses. It provides students with hands-on experience in separation techniques and can help them better understand the principles of chromatography.

5.7. ADVANTAGES OF PAPER CHROMATOGRAPHY:

There are several advantages of using paper chromatography, which include:

Simple and Inexpensive:

Paper chromatography is a simple and inexpensive technique that can be performed with basic laboratory equipment. This makes it accessible to a wide range of users, including students and researchers with limited resources.

High Separation Efficiency:

Paper chromatography can separate a wide range of compounds with high efficiency. This is due to the high surface area of the filter paper and the ability of the solvent to penetrate the paper and interact with the compounds.

Rapid Analysis:

Paper chromatography can provide rapid analysis of a mixture, with results obtained within a few minutes. This makes it useful in time-sensitive applications, such as in clinical or forensic analysis.

Non-Destructive Analysis:

Paper chromatography is a non-destructive technique, which means that the sample can be recovered after the analysis. This is particularly useful in cases where the sample is rare or expensive.

5.8. DISADVANTAGES OF PAPER CHROMATOGRAPHY:

There are also some disadvantages to using paper chromatography, including:

Limited Separation Power:

Paper chromatography has a limited separation power compared to other chromatographic techniques such as high-performance liquid chromatography (HPLC) and gas chromatography (GC). This means that it may not be suitable for complex mixtures.

Limited Quantitative Analysis:

Paper chromatography is not a quantitative technique, meaning that it cannot be used to determine the exact amount of a compound in a mixture.

Sensitivity Issues:

Paper chromatography may not be as sensitive as other chromatographic techniques, meaning that it may not be able to detect low levels of a compound in a mixture.

5.9. LIMITATIONS OF PAPER CHROMATOGRAPHY:

- Large quantity of sample cannot be applied on paper chromatography.
- In quantitative analysis paper chromatography is not effective.
- Complex mixture cannot be separated by paper chromatography.
- Less Accurate compared to HPLC or HPTLC

5.10. SUMMARY:

- To know about in one dimensional or two-dimensional Paper Chromatography.
- To know about the different visualization methods for Paper Chromatography.
- To learn about Identification of amino acids using in Paper chromatography.
- To learn about Identification of sugars using in Paper chromatography.
- To study about applications, advantages, and disadvantages of Paper chromatography.

5.11. SELF ASSESSMENT QUESTIONS:

1) Discuss the one dimensional and two-dimensional techniques in Paper chromatography.
2) Explain the identification of sugars using Paper chromatography.

3) How to identify amino acids using Paper chromatography.

4) Write about detection of visualization methods in Paper chromatography.

5.12. REFERENCE BOOKS:

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4) Instrumental methods of Chemical Analysis by B.K. Sharma, Goel Publish House, Meerut.

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Dr. K. Bala Murali Krishna

LESSON-6

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY: PRINCIPLES AND SEPARATION MODES

6.0. OBJECTIVES:

After studying this lesson, the student will be able to:

- Understand the basic principles of High-Performance Liquid Chromatography (HPLC) and explain the mechanism of separation.
- Distinguish clearly between Normal Phase and Reversed Phase HPLC based on stationary phase, mobile phase, and interaction mechanism.
- Explain the elution behavior, merits, and limitations of NP-HPLC and RP-HPLC.
- Select an appropriate HPLC mode for the separation of polar and non-polar compounds in analytical applications.

STRUCTURE:

6.1 Basic Principles of High-Performance Liquid Chromatography (HPLC)

6.2 Normal Phase and Reversed Phase HPLC

6.2.1. Normal Phase HPLC (NP-HPLC)

6.2.1.1. Nature of the Stationary Phase

6.2.1.2. Nature of the Mobile Phase

6.2.1.3. Mechanism of Separation

6.2.1.4. Elution Behavior

6.2.1.5. Merits

6.2.1.6. Limitations

6.2.2. Reversed Phase HPLC (RP-HPLC)

6.2.2.1. Nature of the Stationary Phase

6.2.2.2. Nature of the Mobile Phase

6.2.2.3. Mechanism of Separation

6.2.2.4. Elution Behavior

6.2.2.5. Advantages and Popularity

6.3 Summary

6.4 Technical Terms

6.5 Self-Assessment Questions

6.6 Reference Books

6.1. BASIC PRINCIPLES OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC):

High Performance Liquid Chromatography (HPLC) is an advanced and widely used **liquid chromatographic separation technique** employed for the **separation, identification, and quantitative estimation of organic and inorganic compounds**. The method is based on the **differential distribution of solute molecules between a liquid mobile phase and a solid stationary phase** packed inside a chromatographic column.

In HPLC, the mobile phase is driven through the column at **high pressure** using a precision mechanical pump. This high-pressure operation makes it possible to use **stationary phases with very small particle sizes**, typically in the micrometer range. The use of small particles greatly increases the surface area available for interaction between the solute and stationary phase, resulting in **high separation efficiency, superior resolution, rapid analysis, and enhanced sensitivity** compared to conventional liquid chromatography.

Separation in HPLC occurs because individual components of a mixture interact with the stationary phase to different extents. These interactions depend on factors such as **polarity, molecular size, functional groups, and chemical nature of the analytes**. Components that interact more strongly with the stationary phase move more slowly through the column and are retained for a longer time, whereas those with weaker interactions elute more rapidly. As a result, the components migrate through the column at different rates and emerge as **well-resolved peaks** in the chromatogram.

Thus, the fundamental principle of HPLC lies in the **controlled partitioning or adsorption of analytes between the mobile and stationary phases under high-pressure conditions**, enabling precise, reproducible, and efficient separations.

6.2. NORMAL PHASE AND REVERSED PHASE HPLC:

High Performance Liquid Chromatography operates mainly in two separation modes- Normal Phase HPLC (NP-HPLC) and Reversed Phase HPLC (RP-HPLC). These two modes differ fundamentally in the nature of the stationary phase, mobile phase polarity, interaction mechanism, and elution behavior. The choice between them depends on the chemical nature of analytes and the separation objective.

6.2.1 Normal Phase HPLC (NP-HPLC):

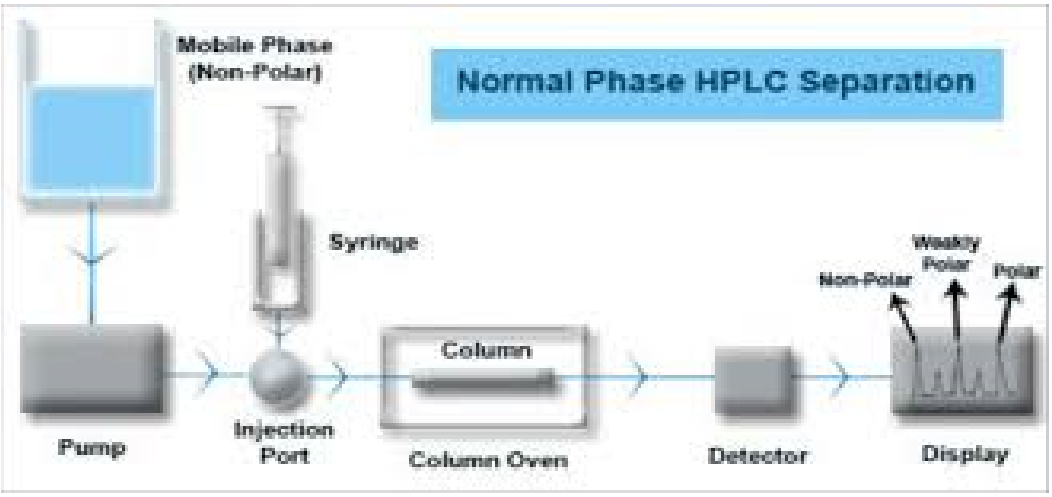

Figure 6.1: Normal Phase HPLC Separation

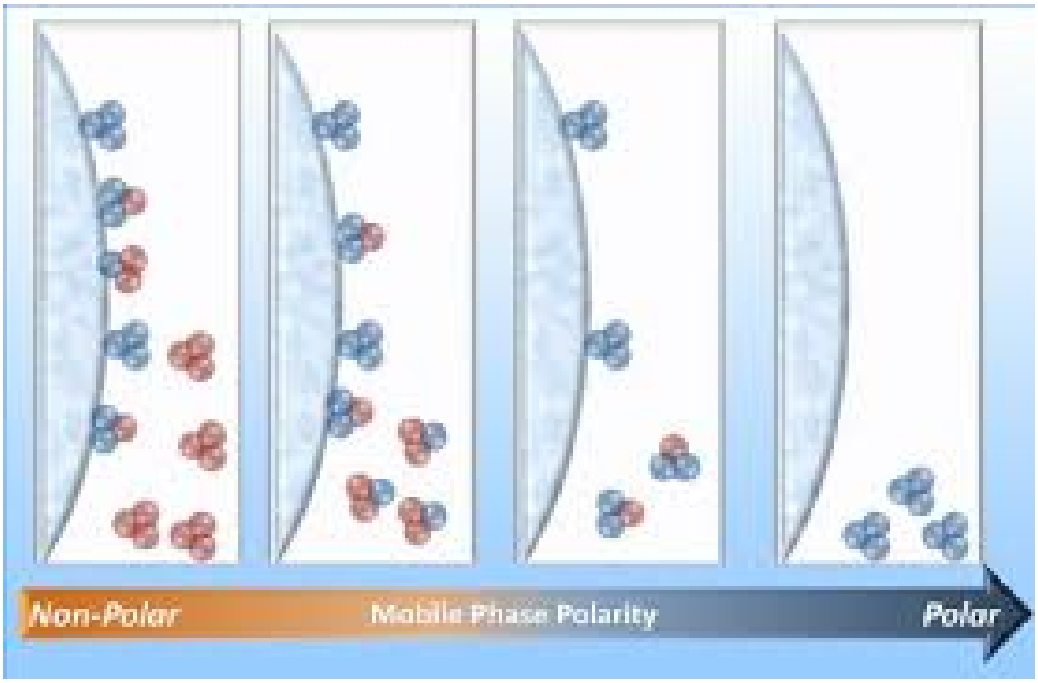

Figure 6.2: Normal Phase Chromatography Separation

6.2.1.1 Nature of the Stationary Phase:

In normal phase HPLC, the stationary phase is **highly polar in nature**. Commonly used materials include **silica gel and alumina**, which possess surface hydroxyl (–OH) groups. These polar functional groups act as **active adsorption sites**, enabling strong interactions with analyte molecules. The presence of these polar sites makes the stationary phase particularly effective in retaining polar compounds.

6.2.1.2 Nature of the Mobile Phase:

The mobile phase employed in NP-HPLC is **non-polar or weakly polar**. Typically, **hexane** is used as the principal solvent due to its non-polar character. To regulate retention time and improve separation, small proportions of polar solvents such as **chloroform, ethyl acetate, or isopropanol** are added as modifiers. Increasing the polarity of the mobile phase enhances its elution strength.

6.2.1.3 Mechanism of Separation:

Separation in NP-HPLC occurs predominantly by an **adsorption mechanism**. Polar analytes interact strongly with the polar stationary phase through:

- Hydrogen Bonding
- Dipole-Dipole Interactions
- Lewis Acid-Base Interactions

Due to these strong interactions, polar compounds are retained longer within the column. In contrast, non-polar analytes exhibit weak interaction with the stationary phase and therefore migrate more rapidly through the column.

6.2.1.4. Elution Behavior:

The elution pattern in normal phase HPLC follows a predictable order:

- **Non-polar compounds elute first**, as they have minimal affinity for the polar stationary phase.
- **Polar compounds elute later** due to stronger adsorption.
- Increasing the polarity of the mobile phase reduces retention time by competing with analytes for adsorption sites on the stationary phase.

6.2.1.5 Merits:

Normal phase HPLC is particularly useful for:

- Separation of **highly polar compounds**
- Resolution of **structural and positional isomers**
- Analysis of compounds poorly retained in reversed phase systems

6.2.1.6 Limitations:

Despite its usefulness, NP-HPLC has certain limitations:

- **Highly sensitive to moisture**, which can affect reproducibility
- **Lower reproducibility** compared to reversed phase HPLC
- **Limited compatibility with aqueous samples**
- Requires careful control of solvent purity and environmental conditions

6.2.2. Reversed Phase HPLC (RP-HPLC):

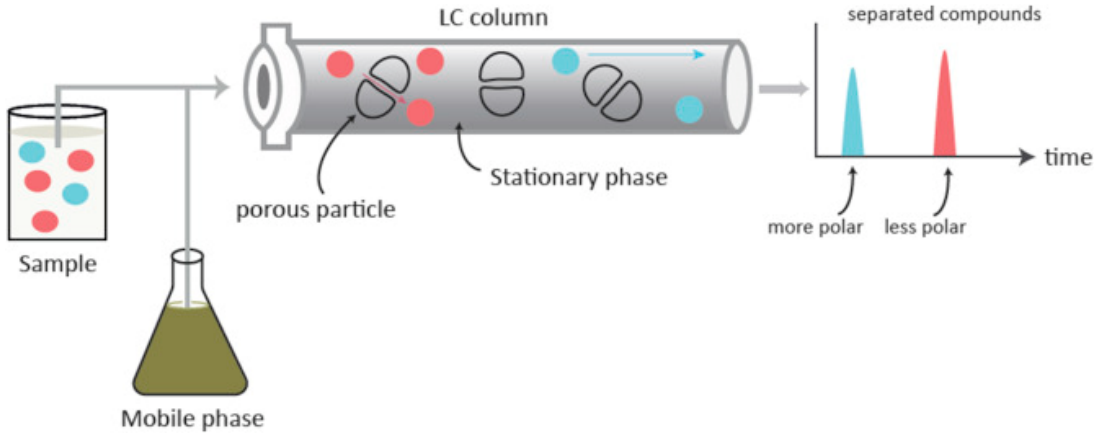

Figure 6.3: Reverse Phase HPLC Separation

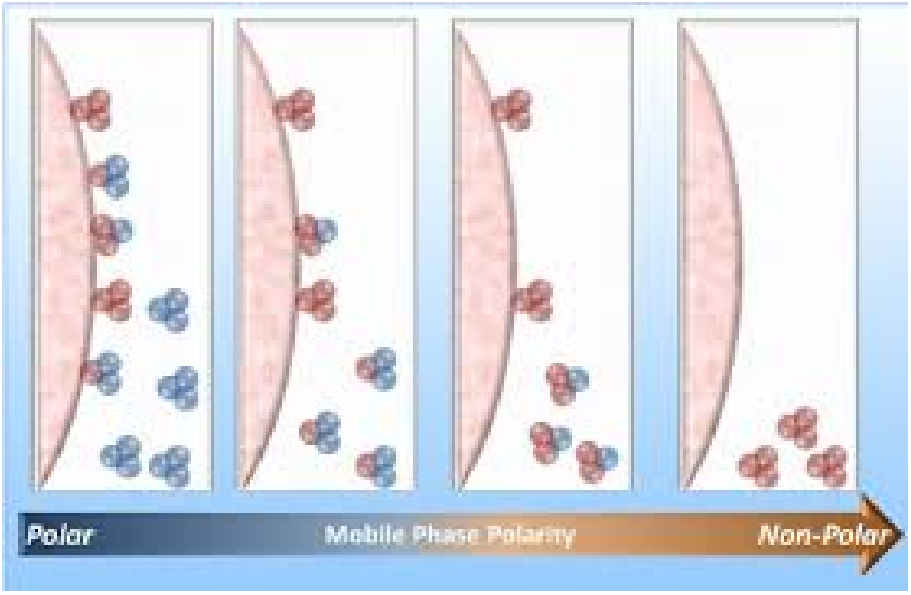

Figure 6.4: Reverse Phase Chromatography Separation

6.2.2.1. Nature of the Stationary Phase:

In reversed phase HPLC, the stationary phase is **non-polar (hydrophobic)** in nature. This is achieved by chemically bonding long hydrocarbon chains such as **C_{18} (octadecylsilane, ODS)** or **C_8** to the surface of silica particles. The bonding process masks the polar silanol (–SiOH) groups of silica, rendering the surface hydrophobic while preserving the **mechanical strength and porosity** of the silica support. Among these, **C_{18} columns** are the most widely used due to their strong hydrophobic character and broad applicability.

6.2.2.2. Nature of the Mobile Phase:

The mobile phase in RP-HPLC is **polar**, typically consisting of **water mixed with organic solvents** such as **methanol or acetonitrile**. Buffer solutions are often added to control the **pH of the mobile phase**, which helps in improving peak shape, reproducibility, and selectivity, especially for ionizable compounds. The polar mobile phase facilitates the movement of polar analytes through the column.

6.2.2.3. Mechanism of Separation:

Separation in RP-HPLC is governed by **hydrophobic (non-polar) interactions** between analyte molecules and the stationary phase.

- **Non-polar analytes** interact strongly with the hydrophobic stationary phase and are therefore retained for longer periods.
- **Polar analytes** have weaker interactions with the stationary phase and preferentially remain in the polar mobile phase, resulting in faster elution.

Thus, the extent of retention increases with the **hydrophobicity of the analyte**, making RP-HPLC particularly effective for separating compounds of varying non-polar character.

6.2.2.4 Elution Behavior:

The elution order in reversed phase HPLC follows a predictable pattern:

- **Polar compounds elute first**, as they have minimal affinity for the non-polar stationary phase.
- **Non-polar compounds elute later** due to stronger hydrophobic interactions.
- Increasing the proportion of the **organic solvent** (methanol or acetonitrile) in the mobile phase reduces retention times by weakening hydrophobic interactions.

6.2.2.5 Advantages and Popularity:

Reversed phase HPLC is the **most widely used chromatographic mode** due to several important advantages:

- Excellent reproducibility and robustness
- Compatibility with aqueous and biological samples
- Applicability to a wide range of organic compounds**, from polar to moderately non-polar**
- Ease of method development and optimization
- Suitability for gradient elution**, enabling separation of complex mixtures**

6.3. SUMMARY:

1) High Performance Liquid Chromatography (HPLC) is an advanced analytical technique used for the **separation, identification, and quantitative estimation** of compounds.
2) Separation in HPLC is based on the **differential interaction of analytes between a liquid mobile phase and a solid stationary phase** under high pressure.
3) Use of small particle size stationary phases provides **high efficiency, better resolution, and faster analysis**.
4) HPLC mainly operates in **Normal Phase and Reversed Phase** modes.
5) Normal Phase HPLC uses a **polar stationary phase and non-polar mobile phase**, with separation governed by adsorption.
6) Reversed Phase HPLC uses a **non-polar stationary phase and polar mobile phase** and is the **most widely used mode** due to its reproducibility and versatility.
7) Selection of HPLC mode depends on the **chemical nature of analytes and analytical requirements**.

6.4. TECHNICAL TERMS:

1) **HPLC** – High pressure liquid chromatographic technique for separation and analysis.
2) **Stationary Phase** – Solid phase packed in the column where separation occurs.
3) **Mobile Phase** – Liquid solvent that carries analytes through the column.

4) **Normal Phase HPLC** – Polar stationary phase with non-polar mobile phase.
5) **Reversed Phase HPLC** – Non-polar stationary phase with polar mobile phase.
6) **Elution** – Movement of analytes out of the column.
7) **Retention Time (t)** – Time taken by a compound to reach the detector.
8) **Silica Gel** – Polar stationary phase used in NP-HPLC.
9) **C_{18} Column** – Non-polar stationary phase used in RP-HPLC.
10) **Resolution** – Measure of separation between two peaks.

6.5. SELF-ASSESSMENTS QUESTIONS:

A. Short Answer Questions:

1) Explain the principle of separation in HPLC.
2) Write the nature of stationary and mobile phases in NP-HPLC.
3) Describe the elution order in normal phase HPLC.
4) Why is RP-HPLC more popular than NP-HPLC?
5) List the merits and limitations of NP-HPLC.

B. Descriptive / Essay Questions:

1) Explain the basic principles of HPLC in detail.
2) Compare Normal Phase and Reversed Phase HPLC.
3) Describe the mechanism of separation in NP-HPLC with merits and limitations.
4) Explain the separation mechanism and elution behavior in RP-HPLC.
5) Discuss the advantages of reversed phase HPLC over normal phase HPLC.

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LESSON-7

INSTRUMENTATION AND METHOD PARAMETERS OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

7.0. OBJECTIVES:

After studying this lesson, the student will be able to:

- Understand the **instrumentation of High-Performance Liquid Chromatography (HPLC)** and the function of each component.
- Explain the **role of column and mobile phase selection** in achieving efficient chromatographic separation.
- Identify factors influencing **resolution, retention time, peak shape, and reproducibility** in HPLC.
- Apply knowledge of **column chemistry and mobile phase composition** for method optimization.

STRUCTURE:

7.1 Instrumentation of High-Performance Liquid Chromatography (HPLC)

7.1.1. Solvent Reservoir

7.1.2. Pump

7.1.3. Sample Injector

7.1.4. Column

7.1.5. Detector

7.1.6. Data System

7.2 Selection of Column and Mobile Phase

7.3 Selection of HPLC Column

7.3.1. Role of the Column in HPLC

7.3.2. Factors Influencing Column Selection

7.3.2.1. Nature and Polarity of Analytes

7.3.2.2. Molecular Weight and Molecular Size

7.3.2.3 Required Resolution and Efficiency

7.3.2.4 Choice of HPLC Mode

7.3.3 Common Types of HPLC Columns

7.3.3.1 Silica Columns (Normal Phase)

7.3.3.2 C_{18} (ODS) Columns (Reversed Phase)

7.3.3.3 C_8 Columns

7.3.3.4 Ion-Exchange Columns

7.3.3.5 Specialized Columns

7.3.3.6 Practical Guidelines for Column Selection

7.4 Selection of Mobile Phase

7.4.1 Role of the Mobile Phase

7.4.2 Key Requirements of a Good Mobile Phase

7.4.2.1 Solubility of Analytes

7.4.2.2 Compatibility with the Detector

7.4.2.3 Low Viscosity and High Purity

7.4.2.4 Chemical Stability and Column Compatibility

7.4.2.5 Commonly Used Mobile Phase Solvents

7.4.2.6 Influence of Mobile Phase Composition

7.5 Summary

7.6 Technical Terms

7.7 Self-Assessment Questions

7.8 Reference Books

7.1. INSTRUMENTATION OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) :

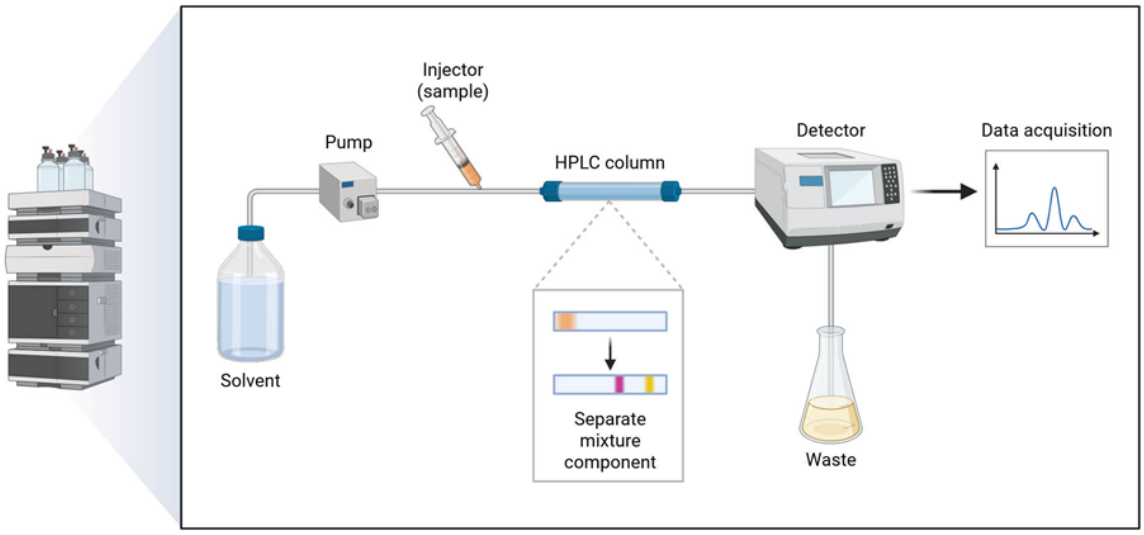

Figure 7.1: Block diagram of HPLC

A typical HPLC system consists of several essential components that work together to achieve **high-resolution separation, detection, and quantification** of analytes. Each component plays a specific role in ensuring accurate and reproducible chromatographic performance.

7.1.1. Solvent Reservoir:

The solvent reservoir is used to **store the mobile phase**, which may consist of a single solvent or a mixture of solvents. The mobile phase must be of **high purity**, as impurities can interfere with detection and damage the column. Before use, solvents are

typically **filtered to remove particulate matter** and **degassed** to eliminate dissolved gases, which could otherwise form bubbles and cause baseline noise or flow instability.

7.1.2. Pump:

The pump is one of the most critical components of the HPLC system. It is responsible for delivering the mobile phase through the column at **high pressure**, typically in the range of **50–400 bar**, while maintaining a **constant and precise flow rate**. A stable flow rate is essential for reproducible retention times and accurate quantitative analysis. Modern HPLC systems use **reciprocating pumps** that provide high pressure capability and minimal flow fluctuations.

7.1.3. Sample Injector:

The sample injector introduces a **small, precise volume of the sample** into the flowing mobile phase. Typical injection volumes range from **5 to 50 µL**. Injection must be highly reproducible to ensure consistent peak areas and reliable quantification. Manual injectors and automated autosamplers are commonly used, with autosamplers preferred for high-throughput and reproducible analysis.

7.1.4. Column:

The column is the **heart of the HPLC system**, as separation of analytes occurs within it. The column is packed with a finely divided **stationary phase**, such as silica or chemically bonded phases (e.g., C_{18}). The interaction between analytes and the stationary phase determines the efficiency, resolution, and selectivity of the separation. Column dimensions, particle size, and surface chemistry significantly influence chromatographic performance.

7.1.5. Detector:

The detector monitors the eluting components as they exit the column and converts their presence into an **electrical signal**. The magnitude of this signal is proportional to the concentration of the analyte. Common HPLC detectors include **UV-Visible, refractive index, fluorescence, and electrochemical detectors**. The choice of detector depends on the chemical nature of the analyte and the required sensitivity.

7.1.6. Data System:

The data system receives the electrical signal from the detector and processes it to produce a **chromatogram**, which is a plot of detector response versus time. The data system enables **peak identification using retention time** and **quantitative analysis using peak area or height**. Modern systems provide advanced software for data acquisition, integration, calibration, and report generation.

7.2. SELECTION OF COLUMN AND MOBILE PHASE:

The selection of an appropriate **HPLC column and mobile phase** is the **most critical step in HPLC method development**, as these two parameters together determine the

selectivity, resolution, sensitivity, peak shape, analysis time, and robustness of the chromatographic method. Even a highly efficient instrument cannot compensate for an improperly chosen column–mobile phase combination. A rational selection ensures that analytes are adequately retained, well resolved from each other and from impurities, and detected with reproducible and symmetrical peaks.

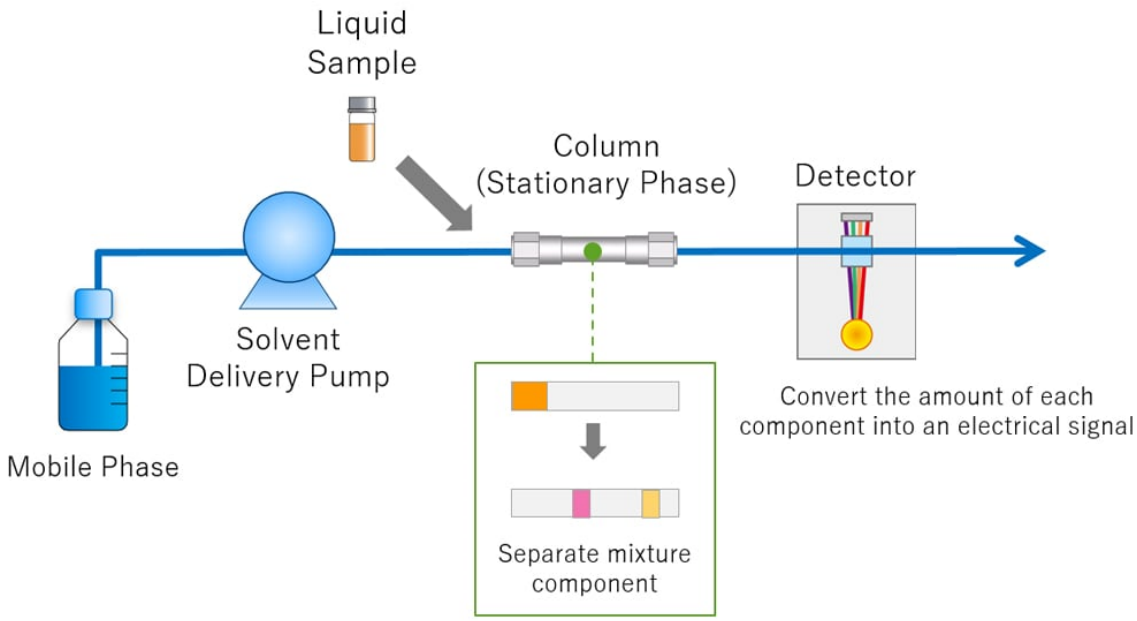

Figure 7.2: Block Diagram of HPLC System

7.3. SELECTION OF HPLC COLUMN:

7.3.1 Role of the Column in HPLC

The column is commonly described as the **"heart" of the HPLC system**, because the actual separation of sample components occurs within it. The column contains a packed **stationary phase**, whose surface chemistry governs how analyte molecules interact, migrate, and separate. Differences in polarity, size, charge, and functional groups of analytes lead to different interaction strengths with the stationary phase, resulting in separation.

7.3.2. Factors Influencing Column Selection:

7.3.2.1. Nature and Polarity of Analytes:

The polarity of the analyte is the primary criterion in column selection.

- **Polar compounds** such as alcohols, sugars, amines, and many natural products interact strongly with polar stationary phases and are best separated using **normal phase HPLC** or **HILIC**.
- **Non-polar to moderately polar compounds**, including most pharmaceuticals, steroids, fatty acids, and aromatic compounds, are best separated using **reversed phase HPLC**, typically on C18 or C8 columns.

7.3.2.2. Molecular Weight and Molecular Size:

The molecular size of the analyte determines the required **pore size** of the stationary phase.

- **Small molecules (< 1000 Da)** are effectively separated using standard analytical columns with pore sizes of ~80–120 Å.
- **Large molecules** such as peptides, proteins, and polymers require columns with **larger pore sizes (100–300 Å)** to allow penetration of analytes into the stationary phase and avoid size-exclusion effects.

7.3.2.3. Required Resolution and Efficiency:

Resolution depends on column **length, particle size, and packing quality**.

- **Smaller particle sizes (3–5 µm)** increase efficiency and resolution but also increase backpressure.
- **Longer columns (150–250 mm)** provide higher resolution and are preferred for complex mixtures.
- **Shorter columns (50–100 mm)** are suitable for rapid screening and routine quality-control analyses.

7.3.2.4. Choice of HPLC Mode:

The chromatographic mode directly dictates column chemistry:

- **Normal phase HPLC** → bare silica or alumina
- **Reversed phase HPLC** → C18, C8, phenyl, polar-embedded phases
- **Ion-exchange HPLC** → cation or anion exchange resins
- **Size-exclusion HPLC** → porous polymer or silica gels
- **Chiral HPLC** → columns containing chiral selectors

7.3.3. Common Types of HPLC Columns:

Figure 7.3: **Key differences between C13 and C8 Columns**

7.3.3.1. Silica Columns (Normal Phase):

Bare silica contains surface silanol (Si–OH) groups and acts as a highly polar stationary phase. Polar compounds are strongly retained, while non-polar compounds elute rapidly. These columns require **strictly anhydrous conditions** and are sensitive to moisture.

7.3.3.2. C_{18} (ODS) Columns (Reversed Phase):

C_{18} columns consist of octadecyl chains bonded to silica and represent the **most widely used HPLC columns**. They are highly hydrophobic and suitable for a wide range of organic compounds, including drugs, metabolites, and environmental pollutants.

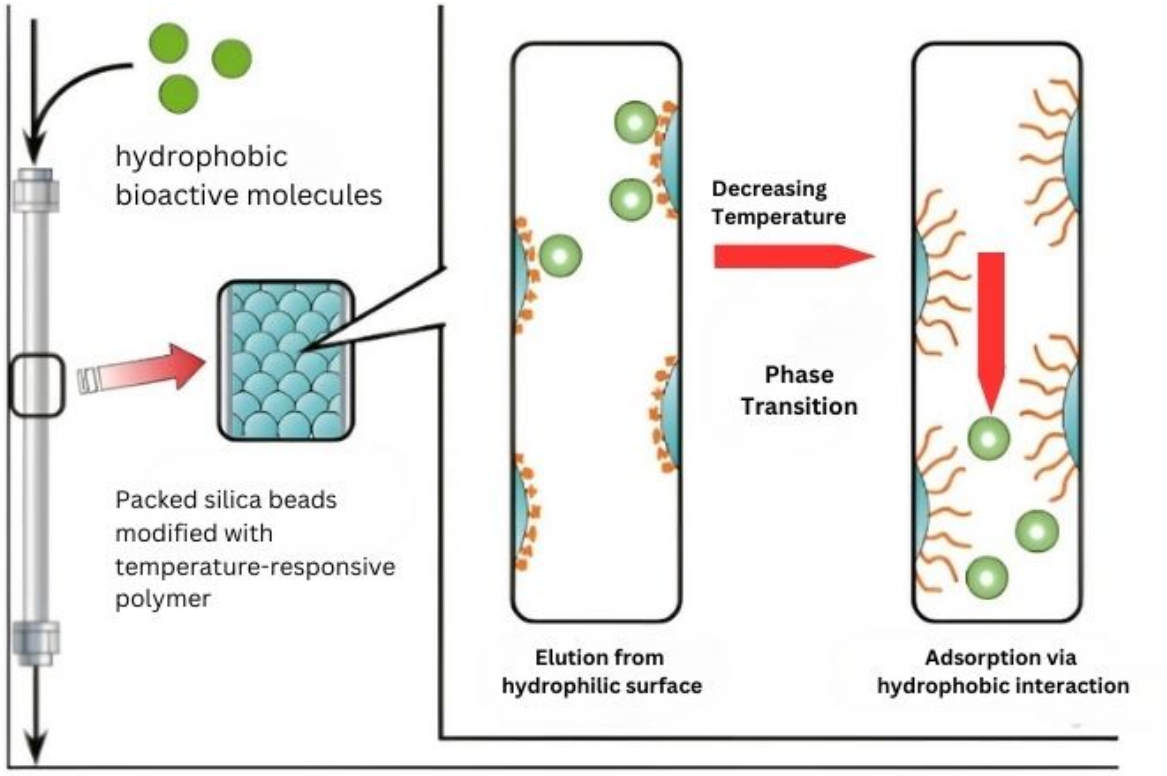

Figure 7.4: C_{18} Reverse Phase Column of HPLC

7.3.3.3. C_8 Columns:

C_8 columns are less hydrophobic than C_{18} columns and are used when analytes are too strongly retained on C_{18} or when faster elution is desired. They are often preferred for peptides and moderately non-polar compounds.

7.3.3.4. Ion-Exchange Columns:

Ion-exchange columns contain charged functional groups and are used for **ionic or ionizable compounds** such as organic acids, bases, amino acids, and nucleotides. Retention depends on charge, pH, and ionic strength of the mobile phase.

7.3.3.5. Specialized Columns:

- **Phenyl columns:** Provide π–π interactions for aromatic compounds.
- **Cyano (CN) and amino (NH_2) columns:** Used for very polar compounds or HILIC separations.
- **Chiral columns:** Separate enantiomers.
- **Size-exclusion columns:** Separate molecules based on size rather than polarity.

7.3.3.6. Practical Guidelines for Column Selection:

- Begin method development with a **C18 column**, as it offers broad applicability.
- For very polar analytes, consider **HILIC or polar-embedded phases**.
- For basic compounds, use **end-capped columns** to minimize peak tailing caused by silanol interactions.
- Always verify **pH and solvent compatibility** of the column.

7.4. SELECTION OF MOBILE PHASE:

7.4.1. Role of the Mobile Phase:

The mobile phase serves as the **transport medium** that carries analytes through the HPLC column. Its composition plays a decisive role in controlling **retention time, selectivity, resolution, peak symmetry, and detector response**. An improperly selected mobile phase may result in poor separation, excessive column backpressure, unstable baselines, or interference with detection.

7.4.2. Key Requirements of a Good Mobile Phase:

7.4.2.1 Solubility of Analytes:

The analyte must be **completely soluble** in the mobile phase to ensure uniform injection and transport through the column. Incomplete solubility can cause **precipitation in the injector or column**, leading to distorted peaks, reduced efficiency, or permanent column damage.

7.4.2.2. Compatibility with the Detector:

The mobile phase must not interfere with the detection system:

1) **UV detectors:** Require solvents with **low UV cut-off**, such as water, methanol, or acetonitrile.
2) **Refractive Index (RI) detectors:** Require **isocratic elution**, as gradients cause baseline drift.
3) **LC–MS systems:** Require **volatile buffers and additives** (e.g., formic acid, acetic acid, ammonium acetate), while non-volatile salts should be avoided.

7.4.2.4. Chemical Stability and Column Compatibility:

The mobile phase should be chemically stable and compatible with the stationary phase, pump seals, tubing, and detector. Extreme pH conditions or aggressive solvents outside the column's recommended limits can degrade the stationary phase and shorten column lifespan.

7.4.2.3. Low Viscosity and High Purity:

Low-viscosity solvents reduce system backpressure, allowing higher flow rates and longer column life. The use of HPLC-grade solvents and high-purity water minimizes baseline noise, ghost peaks, and contamination of the column and detector.

7.4.2.5. Commonly Used Mobile Phase Solvents:

1) **Water:**

   Water is the weak solvent in reversed phase HPLC and is typically used as the aqueous component. It is essential for dissolving polar and ionic compounds.

2) **Methanol:**

   Methanol is stronger than water and is commonly used in reversed phase HPLC. It has higher viscosity than acetonitrile and can give different selectivity.

3) **Acetonitrile (ACN):**

   Acetonitrile is stronger than methanol, has lower viscosity, and often gives sharper peaks and lower backpressure, making it preferred for gradient elution.

4) **Buffered aqueous solutions:**

   Buffered aqueous phases (e.g., phosphate, acetate, formate, citrate) are used to control pH for ionizable compounds, typically in the pH 2–8 range.

7.4.2.6. Influence of Mobile Phase Composition:

1) **Retention Time**

   In reversed phase HPLC, increasing the percentage of organic solvent **decreases retention time**, whereas decreasing organic content increases retention due to stronger analyte–stationary phase interactions.

2) **Resolution**

   Small changes in mobile phase composition (typically **5–10%**) can significantly affect the resolution between closely eluting peaks, making careful optimization essential.

3) **Peak Shape**

   Poor peak symmetry (tailing or fronting) often results from **inappropriate pH, lack of buffering, or secondary interactions**. Proper pH control and buffering improve peak shape and reproducibility.

4) Isocratic vs Gradient Elution

- **Isocratic Elution:** Mobile phase composition remains constant; suitable for simple mixtures with similar polarities.
- **Gradient Elution:** Mobile phase strength increases during the run; essential for complex mixtures containing analytes with a wide range of polarities.

7.5. SUMMARY:

1) HPLC instrumentation consists of essential components such as **solvent reservoir, pump, injector, column, detector, and data system**, all of which work together to achieve accurate separation and detection.
2) The **pump** delivers the mobile phase at high pressure with a constant flow rate, ensuring reproducible retention times.
3) The **column** is the heart of the HPLC system, where separation occurs due to differential interactions between analytes and the stationary phase.
4) Selection of the **HPLC column** depends on analyte polarity, molecular size, required resolution, and chromatographic mode.
5) Common HPLC columns include **silica, C_{18}, C_8, ion-exchange, and specialized columns** such as phenyl and chiral columns.
6) The **mobile phase** plays a critical role in controlling retention time, resolution, peak symmetry, and detector response.
7) Proper selection of solvents, pH, viscosity, and buffer composition is essential for reliable and reproducible analysis.
8) Optimization of column–mobile phase combination is the most important step in **HPLC method development**.

7.6. TECHNICAL TERMS:

1) **HPLC** – A high-pressure liquid chromatographic technique used for separation and analysis.
2) **Solvent Reservoir** – Container used to store the mobile phase.
3) **Pump** – Device that delivers the mobile phase at high pressure and constant flow.
4) **Injector** – Introduces a precise volume of sample into the mobile phase.
5) **Column** – Packed tube containing stationary phase where separation occurs.
6) **Stationary Phase** – Solid material inside the column that retains analytes.
7) **Mobile Phase** – Liquid solvent that transports analytes through the column.
8) **Detector** – Converts eluting analytes into an electrical signal.
9) **Retention Time** – Time taken by an analyte to reach the detector.
10) **Resolution** – Measure of separation between two adjacent peaks.

7.7. SELF-ASSESSMENT QUESTIONS:

A. Short Answer Questions:

1) Define High-Performance Liquid Chromatography.
2) State the function of the HPLC pump.
3) Why is the column calling the heart of the HPLC system?
4) What is the role of degassing the mobile phase?
5) Name any two commonly used HPLC columns.

B. Descriptive / Essay Questions:

1) Describe the instrumentation of HPLC with suitable explanation of each component.
2) Discuss the factors influencing the selection of HPLC columns.
3) Explain the role and requirements of a good mobile phase in HPLC.
4) Describe commonly used mobile phase solvents and their significance.
5) Explain the influence of mobile phase composition on retention time and resolution.

7.8. REFERENCE BOOKS:

1) Skoog, D.A., Holler, F.J., & Crouch, S.R. ***Principles of Instrumental Analysis,* 6th Edition, Cengage Learning.**

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3) Willard, H. H., Merritt, L. L., Dean, J.A., & Settle, F.A. ***Instrumental Methods of Analysis*, 7th Edition, CBS Publishers.**

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5) Snyder, L.R., Kirkland, J.J., & Dolan, J.W. ***Introduction to Modern Liquid Chromatography*, 3rd Edition, Wiley.**

Prof. M. Subba Rao

LESSON-8

HPLC DETECTORS AND RETENTION PARAMETERS

8.0. OBJECTIVES:

After studying this lesson, the student will be able to:

1) Understand the **principle and working of different HPLC detectors**.
2) Select an appropriate **HPLC detector based on analyte properties and sensitivity requirements**.
3) Explain **retention time and key chromatographic performance parameters**.
4) Evaluate **chromatographic efficiency and separation quality** using standard parameters.

STRUCTURE:

8.1 Common Types of HPLC Detectors

8.2 UV-Visible Detector (Most Common)

8.2.1 Principle

8.2.2 Sensitivity and Range

8.2.3 Modes of Operation

8.2.4 Advantages

8.2.5 Limitations

8.2.6 Applications

8.3 Photodiode Array (PDA) / Diode Array Detector (DAD)

8.3.1 Principle

8.3.2 Applications

8.3.3 Advantages

8.3.4 Limitations

8.4 Refractive Index (RI) Detector

8.4.1 Principle

8.4.2 Sensitivity and Range

8.4.3 Advantages

8.4.4 Limitations

8.4.5 Applications

8.5 Fluorescence Detector

8.5.1 Principle

8.5.2 Sensitivity and Selectivity

8.5.3 Advantages

8.5.4 Limitations

8.5.5 Applications

8.6 Electrochemical Detector (ECD)

8.6.1 Principle

8.6.2 Sensitivity and Selectivity

8.6.3 Advantages

8.6.4 Limitations

8.6.5 Applications

8.7 Mass Spectrometric Detector (LC-MS)

8.7.1 Principle

8.7.2 Information Provided

8.7.3 Advantages

8.7.4 Limitations

8.7.5 Applications

8.8 Retention Time (t) and Chromatographic Parameters in HPLC

8.8.1 Retention Time (t_r)

8.8.2 Void Time / Dead Time (t_0)

8.8.3 Capacity Factor (k′)

8.8.4 Resolution (R_s)

8.8.5 Theoretical Plates (N)

8.8.6 Peak Symmetry and Tailing Factor

8.8.7 Significance of Chromatographic Parameters

8.9 Summary

8.10 Technical Terms

8.11 Self-Assessment Questions

8.12 References

8.1. COMMON TYPES OF HPLC DETECTORS:

Detectors in HPLC are essential components that **sense the presence of analytes as they elute from the column** and convert this information into an electrical signal. The detector response is then processed to generate a chromatogram. The choice of detector depends on the **chemical nature of the analyte, required sensitivity, selectivity, and compatibility with the mobile phase**.

8.2. UV–VISIBLE DETECTOR (MOST COMMON):

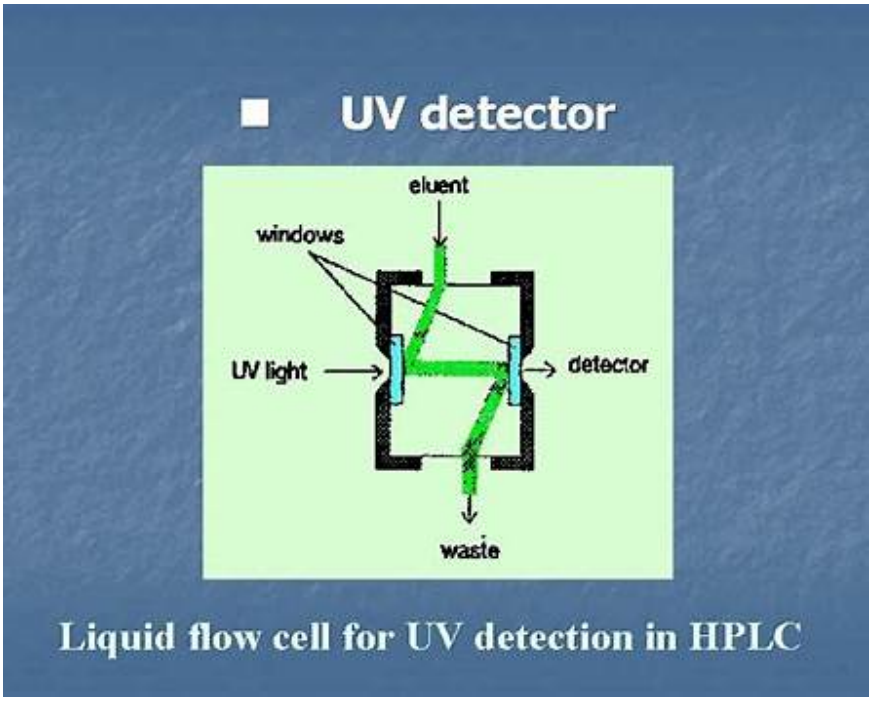


Figure 8.1: Liquid flow for UV detection in HPLC

8.2.1. Principle:

The UV–Visible detector operates by measuring the **absorbance of ultraviolet or visible radiation** by analyte molecules as they pass through a flow cell. Light of a selected wavelength is directed through the eluent, and the decrease in intensity due to absorption by the analyte is measured. According to the **Beer–Lambert law**, absorbance is directly proportional to analyte concentration.

8.2.2. Sensitivity and Range:

The UV detector offers **good sensitivity**, typically in the **nanogram to microgram range**, and a wide linear dynamic range. This makes it suitable for both qualitative identification and quantitative analysis.

8.2.2.1. Modes of Operation:

- **Fixed wavelength mode** is used when the analyte has a strong and known absorption maximum (e.g., 254 nm for aromatic compounds).
- **Variable wavelength mode** allows the operator to select the most suitable wavelength for maximum sensitivity.
- **Multi-wavelength mode** enables simultaneous monitoring at more than one wavelength, useful for multi-component mixtures.

8.2.3. Advantages:

The UV detector is simple, reliable, non-destructive, and **fully compatible with gradient elution**, making it the most widely used HPLC detector.

8.2.4. Limitations:

Only compounds containing **UV-absorbing chromophores** can be detected. In addition, the mobile phase must be transparent at the selected wavelength.

8.2.5. Applications:

Widely used for pharmaceuticals, aromatic compounds, conjugated systems, and natural products.

8.3. PHOTODIODE ARRAY (PDA) / DIODE ARRAY DETECTOR (DAD):

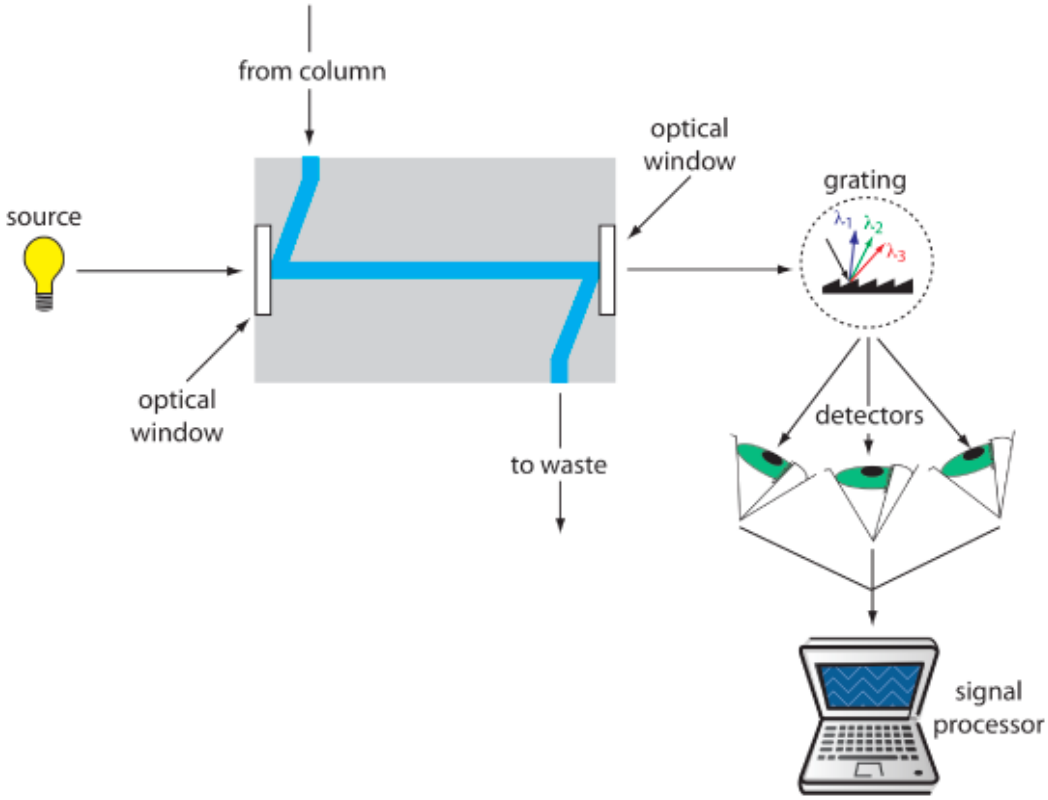


Figure 8.2: Schematic Diagram of a Flow Cell for a Detector Equipped with a Diode Array Spectrometer

8.3.1. Principle:

The PDA/DAD detector is an advanced UV detector that records **absorbance across a wide range of wavelengths simultaneously**. Polychromatic light passes through the flow cell, and the transmitted light is dispersed onto an array of photodiodes, enabling acquisition of a complete UV spectrum for each eluting peak.

8.3.2. Applications:

This detector is particularly useful for **peak purity analysis**, identification of unknown compounds through spectral matching, and selection of optimal detection wavelengths during method development.

8.3.4. Advantages:

PDA/DAD provides **spectral as well as chromatographic information**, allows post-run data analysis at different wavelengths, and is compatible with gradient elution.

8.3.5. Limitations:

Its sensitivity is slightly lower than that of single-wavelength UV detectors, and it requires more advanced data processing.

8.4. REFRACTIVE INDEX (RI) DETECTOR:

8.4.1. Principle:

The RI detector measures the **change in refractive index** of the mobile phase caused by the presence of an analyte. The detector compares the refractive index of the pure mobile phase with that of the eluent containing the analyte, producing a signal proportional to concentration.

8.4.2. Sensitivity and Range:

The RI detector is a **universal detector**, responding to most compounds, but it has **relatively low sensitivity** compared to UV or fluorescence detectors.

8.4.3. Advantages:

It is non-destructive and useful for compounds that **do not absorb UV light**, such as sugars and polymers.

8.4.4. Limitations:

The RI detector is **highly sensitive to temperature and mobile phase changes** and therefore cannot be used with gradient elution. Strict temperature control is required.

8.4.5. Applications:

Commonly used for carbohydrates, alcohols, lipids, and polymer analysis.

8.5. FLUORESCENCE DETECTOR:

8.5.1. Principle:

The fluorescence detector works by exciting analyte molecules at a specific wavelength and measuring the **emitted fluorescence** at a longer wavelength. Only compounds capable of fluorescence produce a signal.

8.5.2. Sensitivity and Selectivity:

This detector offers **very high sensitivity**, often in the **picogram to nanogram range**, and excellent selectivity.

8.5.3. Advantages:

It provides extremely low detection limits, low background interference, and is compatible with gradient elution.

8.5.4. Limitations:

Only fluorescent compounds can be detected, and many analytes require **derivatization** to introduce a fluorescent group.

8.5.5. Applications:

Used in trace analysis of PAHs, vitamins, amino acids, drugs, and biomolecules.

8.6. ELECTROCHEMICAL DETECTOR (ECD):

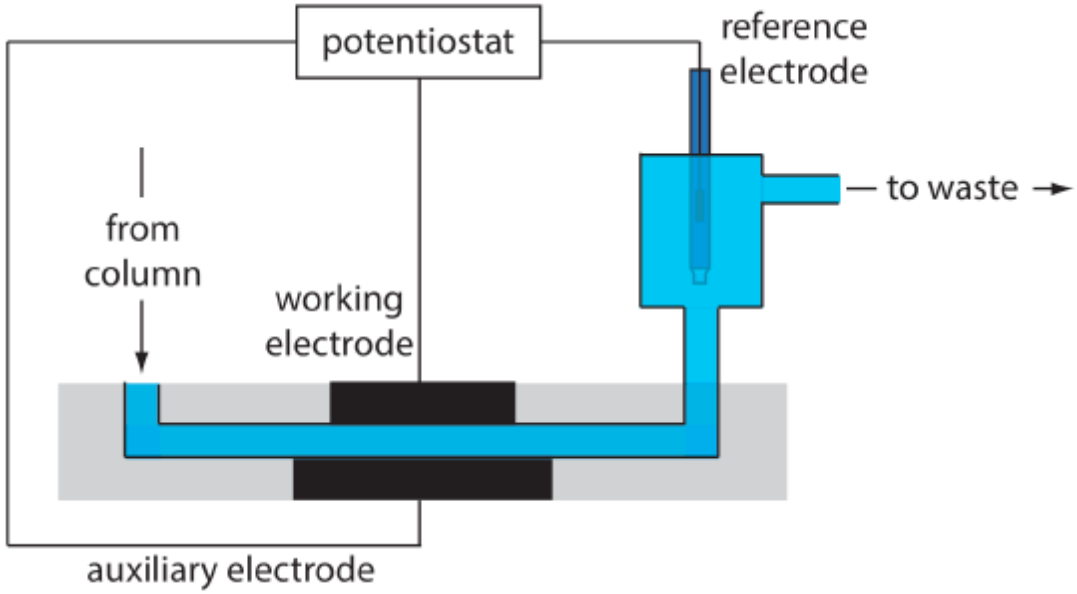


Figure 8.3: Schematic Diagram Showing a Flow Cell for An Amperometric Electrochemical Detector

8.6.1. Principle:

The electrochemical detector measures the **current generated by oxidation or reduction** of electroactive analytes at an electrode surface maintained at a fixed potential.

8.6.2. Sensitivity and Selectivity:

ECD offers **very high sensitivity** and selectivity for compounds that undergo electrochemical reactions.

8.6.3. Advantages:

Highly sensitive and selective when operating conditions are optimized.

8.6.4. Limitations:

Only electroactive compounds can be detected, and electrode fouling may occur with complex samples.

8.6.5. Applications:

Widely used for catecholamines, phenols, neurotransmitters, and antioxidants.

8.7. MASS SPECTROMETRIC DETECTOR (LC–MS):

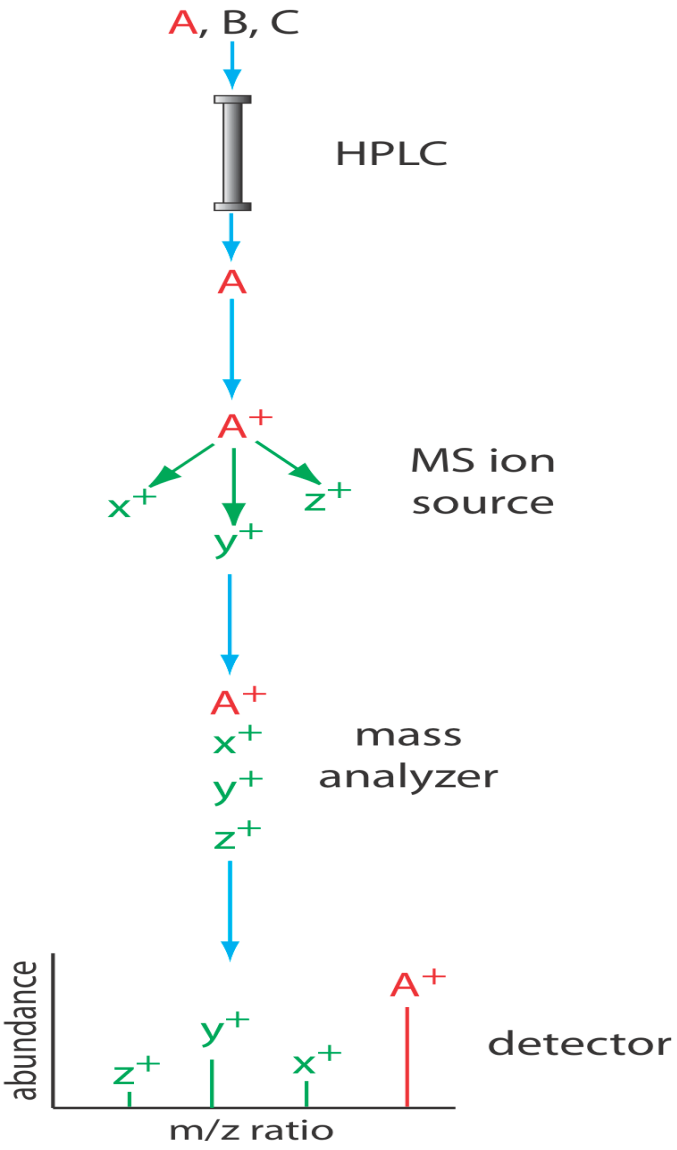


Figure 8.4: Block Diagram of an HPLC–MS. A Detector Counts the Ions and Displays the Mass Spectrum

8.7.1. Principle:

In LC–MS, the eluent from the HPLC column is introduced into a mass spectrometer, where analytes are ionized and separated based on their **mass-to-charge (m/z) ratio**.

8.7.2. Information Provided:

LC–MS provides **molecular weight, structural information, and highly selective quantitative data**, especially when tandem MS is used.

8.7.3. Advantages:

It offers unparalleled identification power, very high sensitivity, and compatibility with gradient elution.

8.7.4. Limitations:

The technique is expensive, complex, and requires **MS-compatible (volatile) mobile phases**.

8.7.5. Applications:

Extensively used in drug discovery, metabolomics, impurity profiling, environmental analysis, and advanced research.

8.8. RETENTION TIME (T) AND CHROMATOGRAPHIC PARAMETERS IN HPLC

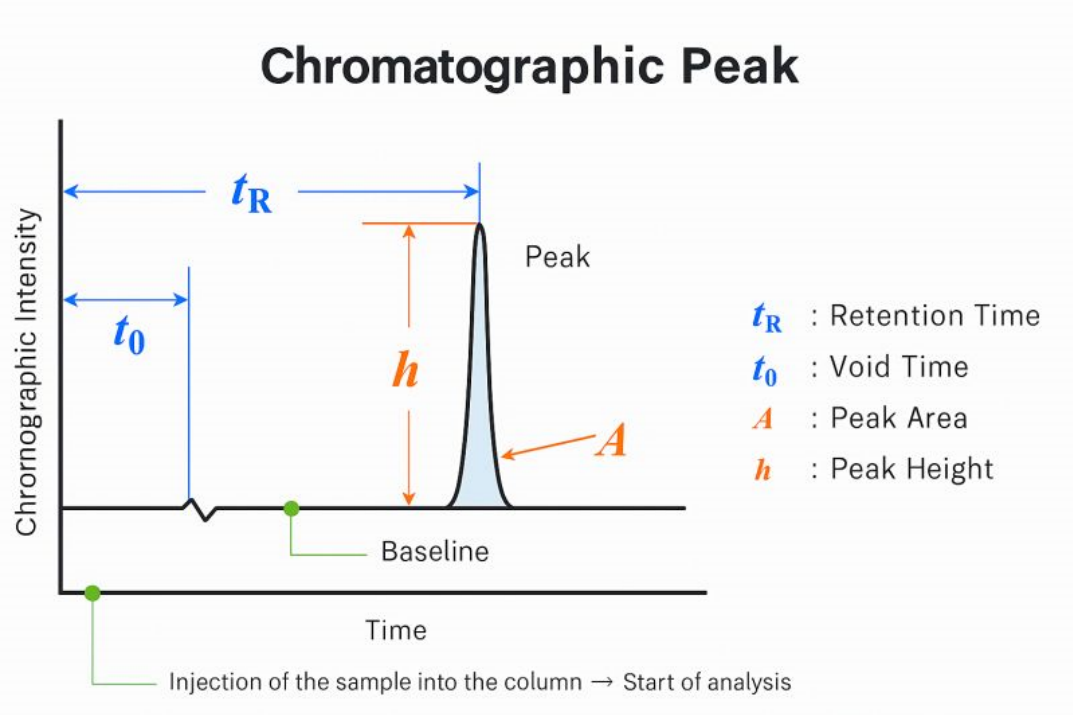


Figure 8.5: Chromatographic Peak and Retention Time Diagram

8.8.1. Retention Time (t):

Retention time (t_r) is defined as the **time elapsed between the injection of a sample and the appearance of the maximum (apex) of the analyte peak at the detector**. It is measured directly from the chromatogram.

Under a fixed set of chromatographic conditions-such as **column type, mobile phase composition, flow rate, and temperature**-each compound exhibits a **characteristic and reproducible retention time**. Therefore, retention time is primarily used for the **qualitative identification of compounds** by comparison with standards analyzed under identical conditions.

8.8.2. Void Time / Dead Time (t_0):

Void time, also called **dead time (t_0)**, is the **retention time of an unretained compound** that passes through the column without interacting with the stationary phase. It represents the time taken by the **mobile phase alone** to travel from the injector to the detector.

In reversed phase HPLC, compounds such as **uracil** are commonly used to measure t_0. Void time provides an important reference point for evaluating the degree of retention of analytes.

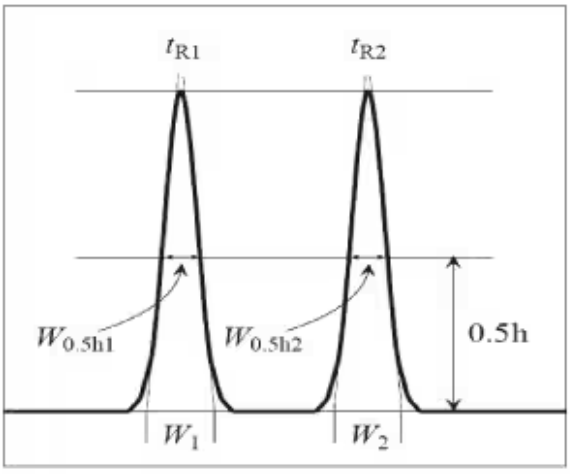
8.8.3. Capacity Factor (k′):

The capacity factor (k′), also known as the **retention factor**, is a measure of how strongly an analyte is retained by the stationary phase relative to the mobile phase. It is defined as:

$$k\prime = \frac{t_r - t_0}{t_0}$$

A higher k′ value indicates greater retention. For efficient and practical HPLC separations, **k′ values in the range of 2–10** are considered ideal, as they provide a good balance between resolution and analysis time. Very low k′ values result in poor separation, while very high values lead to excessively long run times.

8.8.4. Resolution (R_s):



t_{R1}, t_{R2} : Retention time for each peak ($t_{R1} < t_{R2}$)
$W_{0.5h1}$, $W_{0.5h2}$: Full width at half maximum (FWHM) of each peak
W_1, W_2 : Width of each peak
Fig. 1 Two Adjacent Peaks

Figure 8.6: Chromatogram Showing Two Adjacent Peaks (Resolution Diagram)

Resolution (R_s) is a quantitative measure of the **degree of separation between two adjacent chromatographic peaks**.

It is given by:

$$R_s = \frac{2(t_{r2} - t_{r1})}{w_1 + w_2}$$

where:

- t_{r1} and t_{r2} are the retention times of two adjacent peaks
- w_1 and w_2 are the corresponding peak widths

A resolution value of $\mathbf{R_s \geq 1.5}$ indicates **baseline separation**, meaning the two peaks are completely resolved without overlap. Resolution is a critical parameter in method development and validation.

8.8.5. Theoretical Plates (N):

The number of theoretical plates (N) is a measure of **column efficiency**. A higher N value indicates better separation efficiency and narrower peaks. It reflects how effectively the column can separate closely related compounds and is influenced by particle size, column length, and packing quality.

$$N = 16\left(\frac{t_R}{w}\right)^2$$

where **ω** is the peak width at the base (in time units).

8.8.6. Peak Symmetry and Tailing Factor (T_f):

Peak symmetry describes the **shape of chromatographic peaks**. Ideally, peaks should be symmetrical and Gaussian in shape. Peak asymmetry is often expressed using the **tailing factor**, which indicates peak tailing or fronting.

$$T_f = \frac{w_{0.05}}{2a}$$

where:

- $\omega_{0.05}$: peak width at 5% of peak height,
- 2a: distance from the peak front to the peak back at 5% height.

Good peak symmetry is essential for **accurate quantification**, reproducible retention times, and reliable method validation. Poor peak shape may arise from inappropriate pH, secondary interactions with the stationary phase, or column overloading.

8.8.6.1. Acceptable Range:

- For most methods, T_f is acceptable in the range 0.8–1.5.
- Excessive tailing ($T_f > 2.0$) can reduce resolution and affect quantitation accuracy.

8.8.7. Overall Significance:

Together, **retention time, void time, capacity factor, resolution, theoretical plates, and peak symmetry** provide a comprehensive description of chromatographic performance. These parameters are essential for **method development, optimization, system suitability testing, and validation** in HPLC.

8.9. SUMMARY:

1) Detectors in HPLC sense analytes eluting from the column and convert their presence into an **electrical signal**, which is processed to obtain a chromatogram.
2) The **UV–Visible detector** is the most widely used detector due to its simplicity, reliability, and compatibility with gradient elution.
3) **PDA/DAD detectors** provide spectral information across multiple wavelengths, aiding in peak purity and compound identification.
4) The **Refractive Index detector** is a universal detector suitable for non-UV-absorbing compounds but is limited to isocratic elution.
5) **Fluorescence detectors** offer very high sensitivity and selectivity for fluorescent compounds.
6) **Electrochemical detectors** are highly sensitive for electroactive analytes such as phenols and neurotransmitters.
7) **Mass spectrometric detectors (LC–MS)** provide molecular weight and structural information with exceptional sensitivity.
8) Retention parameters such as **retention time, dead time, capacity factor, resolution, theoretical plates, and peak symmetry** are essential for assessing chromatographic performance.
9) These parameters are crucial for **method development, optimization, system suitability testing, and validation**.

8.10. TECHNICAL TERMS:

1) **Detector** – Device that converts analyte concentration into an electrical signal.
2) **UV–Visible Detector** – Measures absorbance of UV/visible light by analytes.
3) **PDA/DAD** – Detector that records full UV spectra simultaneously.
4) **Refractive Index Detector** – Measures change in refractive index of the eluent.
5) **Fluorescence Detector** – Detects emitted light from excited analytes.
6) **Electrochemical Detector** – Measures current from redox reactions of analytes.
7) **LC–MS** – HPLC coupled with mass spectrometry for molecular identification.
8) **Retention Time (t)** – Time taken by an analyte to reach the detector.
9) **Dead Time (t_0)** – Time taken by an unretained compound to elute.
10) **Capacity Factor (k')** – Measure of analyte retention relative to mobile phase.
11) **Resolution (R_s)** – Degree of separation between adjacent peaks.
12) **Theoretical Plates (N)** – Measure of column efficiency.
13) **Tailing Factor (Tf)** – Measure of peak symmetry.

8.11. SELF-ASSESSMENT QUESTIONS:

A. Short Answer Questions

1) What is the role of a detector in HPLC?
2) Why is the UV–Visible detector most commonly used?
3) Define retention time.
4) What is dead time in HPLC?
5) State the significance of capacity factor.

B. Descriptive / Essay Questions:

1) Describe the principle, advantages, and limitations of the UV–Visible detector.
2) Explain the working and applications of PDA/DAD detectors.
3) Discuss the principle and applications of RI and fluorescence detectors.
4) Describe the working of an electrochemical detector.
5) Explain the principle and advantages of LC–MS detection.
6) Define retention time, dead time, capacity factor, and resolution.
7) Discuss theoretical plates and peak symmetry with their significance.
8) Explain the importance of chromatographic parameters in method validation.

8.12. REFERENCE BOOKS:

1) **Skoog, D.A., Holler, F.J., & Crouch, S.R.** *Principles of Instrumental Analysis,* 6th Edition, Cengage Learning.
2) **Skoog, D.A., West, D.M., Holler, F.J., & Crouch, S.R.** *Fundamentals of Analytical Chemistry,* 9th Edition, Cengage Learning.
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Prof. M. Subba Rao

LESSON-9

APPLICATIONS OF HPLC IN ORGANIC CHEMISTRY AND METHOD DEVELOPMENT

9.0. OBJECTIVES:

After studying this lesson, the student will be able to:

1) Understand the **applications of HPLC in organic chemistry** for separation, identification, and quantitative analysis.
2) Explain the use of HPLC in **separation of complex organic mixtures**, isomers, and reaction products.
3) Apply HPLC techniques for **identification and quantitative estimation** of organic compounds.
4) Understand the **concepts and systematic steps involved in HPLC method development**.
5) Appreciate the role of HPLC in **pharmaceutical, natural product, and synthetic organic chemistry**

STRUCTURE:

9.1. Applications in Organic Chemistry

9.2. Separation of Organic Mixtures

9.2.1. Isolation of Reaction Products

9.2.2. Purification of Synthetic Intermediates

9.2.3. Separation of Isomers

9.2.4. Complex Mixtures

9.3. Identification of Organic Compounds

9.3.1. Retention Time Comparison with Standards

9.3.2. Use of PDA/DAD for Spectral Confirmation

9.3.3. Structural Confirmation by LC–MS

9.4. Quantitative Estimation of Organic Compounds

9.4.1. External Standard Method

9.4.2. Internal Standard Method

9.4.3. Validation Parameters

9.5. Specific Applications in Organic Chemistry

9.5.1. Analysis of Pharmaceuticals

9.5.2. Quality Control of Natural Products

9.5.3. Monitoring of Reaction Progress

9.5.4. Purity Assessment of Organic Compounds

9.6. Concepts of HPLC Method Development

9.6.1. Introduction

9.6.2. Purpose of HPLC Method Development

9.6.3. Selection of Chromatographic Mode

9.6.4. Selection of Column

9.6.5. Selection of Mobile Phase and Ph

9.6.6. Optimization of Flow Rate and Temperature

9.6.7. Selection of Detector

9.6.8. Desired Outcomes of Method Development

9.7. Summary

9.8. Technical Terms

9.9. Self-Assessment Questions

9.10. References Textbooks

9.1. APPLICATIONS IN ORGANIC CHEMISTRY:

High Performance Liquid Chromatography (HPLC) is an indispensable tool in modern organic chemistry laboratories. It is widely used not only for routine analysis but also for method development, reaction monitoring, and quality control of synthetic and natural organic compounds. Its high resolution, sensitivity, and compatibility with a wide range of solvents and detectors make it ideal for solving complex problems in organic synthesis, pharmaceutical chemistry, and natural product research.

9.2. SEPARATION OF ORGANIC MIXTURES:

HPLC is routinely employed to separate complex mixtures of organic compounds, which is essential in both research and industrial settings.

9.2.1. Isolation of Reaction Products:

After a synthetic reaction, the crude mixture often contains the desired product along with starting materials, by-products, and side products. HPLC allows selective isolation of the target compound, especially when combined with preparative HPLC systems. This is particularly useful for high-value compounds such as pharmaceuticals, fine chemicals, and natural products.

9.2.2. Purification of Synthetic Intermediates:

Many multi-step syntheses involve intermediates that are difficult to purify by conventional methods (e.g., recrystallization, distillation). HPLC provides a gentle, efficient way to purify these intermediates, especially when they are thermally sensitive or have similar boiling points/polarities.

9.2.3. Separation of Isomers:

HPLC is highly effective in resolving various types of isomers:

- **Structural Isomers**: e.g., ortho, meta, and para substituted aromatic compounds, which often have very similar physical properties but different retention behavior on reversed phase or normal phase columns.
- **Stereoisomers:**

  1) Diastereomers can usually be separated on standard reversed phase or normal phase columns.

  2) Enantiomers require chiral stationary phases (chiral columns) that interact differentially with each enantiomer, allowing their separation and quantification.

9.2.4. Complex Mixtures:

HPLC is used to separate mixtures such as crude extracts, reaction mixtures, and degradation products, where components may differ only slightly in polarity, size, or charge.

9.3. IDENTIFICATION OF ORGANIC COMPOUNDS:

HPLC plays a key role in the identification of organic compounds, especially when combined with spectral detectors.

9.3.1. Retention Time Comparison with Standards:

Under identical chromatographic conditions (same column, mobile phase, flow rate, temperature), a compound elutes at a characteristic retention time (tR). By comparing the tR of an unknown peak with that of an authentic reference standard analyzed under the same conditions, tentative identification can be made. This is commonly used in:

- Identification of known APIs (Active Pharmaceutical Ingredients).
- Detection of known impurities or degradation products in drug substances and products.

9.3.2. Use of PDA/DAD for Spectral Confirmation:

The Photodiode Array (PDA) or Diode Array Detector (DAD) records the UV-Vis spectrum of each peak across its elution profile. This allows:

- Confirmation that a peak corresponds to the expected compound by matching its spectrum with that of a reference standard.
- Detection of co-elution (peak impurity) by comparing spectra at the front, apex, and tail of the peak.
- Selection of the optimal detection wavelength for maximum sensitivity and selectivity.

9.3.3. Structural Confirmation by LC-MS:

When coupled with mass spectrometry (LC-MS), HPLC provides:

- Molecular weight information (from $[M+H]^+$, $[M–H]^-$, or other ions).
- Fragmentation patterns (in MS/MS mode) that give clues about the structure.
- Confirmation of the identity of unknown peaks, impurities, degradation products, and metabolites.

9.4. QUANTITATIVE ESTIMATION OF ORGANIC COMPOUNDS:

HPLC is one of the most reliable techniques for the quantitative analysis of organic compounds in complex matrices.

9.4.1. External Standard Method:

- A series of standard solutions of known concentrations of the analyte are injected, and a calibration curve (peak area vs. concentration) is constructed.
- The peak area of the analyte in the sample is then compared with the calibration curve to determine its concentration.
- This method is simple and widely used for routine analysis of APIs, impurities, and natural products.

9.4.2. Internal Standard Method:

- A known amount of a suitable internal standard (a compound not present in the sample, with similar chromatographic behavior) is added to both standards and samples.
- The ratio of the analyte peak area to the internal standard peak area is used to construct the calibration curve.
- This method corrects for variations in injection volume, instrument response, and sample preparation, leading to improved precision and accuracy.
- It is particularly useful for biological samples, stability studies, and bioanalytical applications.

9.4.3. Validation Parameters:

For reliable quantification, the method is validated for:

- Linearity, range, accuracy, precision (repeatability, intermediate precision), LOD (Limit of Detection), LOQ (Limit of Quantitation), and robustness.

9.5. SPECIFIC APPLICATIONS IN ORGANIC CHEMISTRY:

HPLC finds diverse and critical applications in various areas of organic chemistry:

9.5.1 Analysis of Pharmaceuticals:

- APIs (Active Pharmaceutical Ingredients): Quantification of the main component in drug substances and formulations.
- Impurity profiling: Identification and quantification of process-related impurities, residual solvents, and related substances.
- Degradation studies: Monitoring forced degradation (acid, base, oxidation, light, heat) to identify degradation products and establish stability-indicating methods.

9.5.2. Quality Control of Natural Products:

- Alkaloids, flavonoids, terpenes, glycosides, and other phytoconstituents: HPLC is used to standardize herbal extracts and formulations by quantifying marker compounds.
- Purity assessment: Ensuring that natural product isolates meet purity criteria for further biological or chemical studies.
- Batch-to-batch consistency: Checking the composition of herbal products across different batches.

9.5.3. Monitoring of Reaction Progress:

- HPLC is used to follow the course of a reaction by analyzing aliquots taken at different time intervals.
- It helps in:
  - Determining reaction completion.
  - Identifying intermediates and by-products.
  - Optimizing reaction conditions (time, temperature, catalyst, solvent).
- This is especially valuable in asymmetric synthesis, catalysis, and multi-step sequences.

9.5.4. Purity Assessment of Organic Compounds:

- HPLC is routinely used to determine the purity of synthesized compounds (e.g., >95%, >98%, or >99%).
- It can detect low-level impurities (0.05–0.1%) that may not be visible by TLC or NMR, which is crucial for high-purity requirements in pharmaceuticals and materials chemistry.

9.6. CONCEPTS OF HPLC METHOD DEVELOPMENT:

9.6.1. Introduction:

HPLC method development is a **systematic and scientific approach** used to establish chromatographic conditions that provide reliable separation, identification, and quantitative estimation of analytes. It is an essential step in analytical chemistry, particularly for pharmaceutical, environmental, food, and biochemical analysis, where accuracy, precision, and reproducibility are critical.

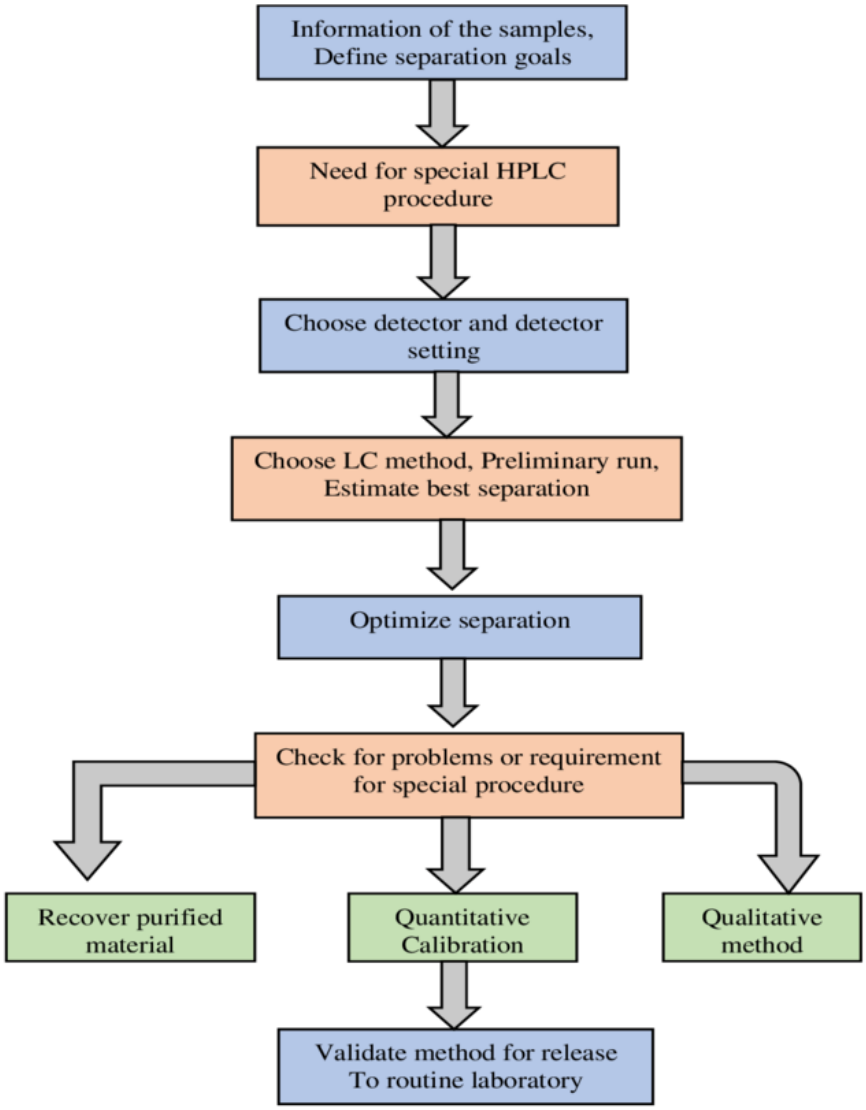

Figure 9.1: Steps involved in HPLC Method Development

9.6.2. Purpose of HPLC Method Development:

The main purpose of method development is to design an HPLC procedure that is **efficient, reproducible, and suitable for routine analysis**. A well-developed method ensures consistent performance and minimizes experimental variability during repeated use.

9.6.3. Selection of Chromatographic Mode:

The first step in HPLC method development is the **selection of an appropriate chromatographic mode**, based on the chemical nature of the analytes.

- **Reversed Phase HPLC (RP-HPLC)** is most commonly selected due to its wide applicability, compatibility with aqueous samples, and good reproducibility.
- **Normal Phase HPLC (NP-HPLC)** is chosen for highly polar compounds that are poorly retained in reversed phase systems.

The correct choice of mode provides the foundation for effective separation.

9.6.4. Selection of Column:

The column plays a crucial role in method development, as separation occurs within it.

- **Stationary phase chemistry** (C_{18}, C_8, silica, ion-exchange, etc.) is selected based on analyte polarity and functionality.
- **Column length and particle size** influence resolution and efficiency.
- **Pore size** is selected according to the molecular size of the analytes.

In practice, method development often begins with a **standard C_{18} column**, and modifications are made if separation is unsatisfactory.

9.6.5. Selection of Mobile Phase and pH:

The mobile phase controls **retention time, selectivity, resolution, and peak shape**.

- Choice of organic solvent (methanol or acetonitrile) and its proportion with water determines elution strength.
- **pH optimization** is essential for ionizable compounds to improve peak symmetry and reproducibility.
- Buffered mobile phases help maintain stable chromatographic conditions.

Proper mobile phase selection is critical for achieving consistent and reliable separations.

9.6.6. Optimization of Flow Rate and Temperature:

Flow rate and column temperature significantly influence analysis time and efficiency.

- Higher flow rates reduce run time but may compromise resolution.
- Column temperature affects solvent viscosity and analyte–stationary phase interactions.

Optimization ensures a balance between **speed and chromatographic performance**.

9.6.7. Selection of Detector:

The detector is selected based on the **chemical nature of analytes, required sensitivity, and compatibility with the mobile phase**.

- UV-Visible detectors are most commonly used.
- PDA, fluorescence, electrochemical, or mass spectrometric detectors are selected when higher sensitivity or structural information is required.

Proper detector selection ensures accurate qualitative and quantitative analysis.

9.6.8. Desired Outcomes of Method Development:

A successfully developed HPLC method should provide:

- **Good resolution** between analytes and impurities
- **Symmetrical and well-defined peaks**

- **Reasonable analysis time**
- **High reproducibility and robustness**

Such a method is suitable for routine analysis and further method validation.

9.7. SUMMARY:

1) HPLC is an indispensable analytical technique in organic chemistry for **separation, identification, and quantitative estimation** of compounds.
2) It is widely used for **separating reaction mixtures, purifying synthetic intermediates, and resolving isomers**, including enantiomers using chiral columns.
3) Identification of organic compounds is achieved by **retention time comparison**, supported by **PDA/DAD spectral analysis** and **LC–MS molecular weight confirmation**.
4) Quantitative estimation is performed using **external standard and internal standard methods**, providing high accuracy and precision.
5) HPLC is extensively applied in **pharmaceutical analysis, impurity profiling, degradation studies, reaction monitoring, and purity assessment**.
6) HPLC method development is a **systematic optimization process** involving selection of chromatographic mode, column, mobile phase, operating conditions, and detector.
7) A well-developed HPLC method provides **good resolution, symmetrical peaks, reasonable analysis time, and reproducible results**.

9.8. TECHNICAL TERMS (ONE-LINE GLOSSARY):

1) **HPLC** – A high-resolution liquid chromatographic technique operated under high pressure.
2) **Retention Time (t)** – Time taken by an analyte to reach the detector after injection.
3) **Preparative HPLC** – HPLC used for isolation and purification of compounds.
4) **Chiral Column** – A stationary phase used to separate enantiomers.
5) **PDA/DAD Detector** – Detector that records UV–Vis spectra of eluting peaks.
6) **LC–MS** – Hyphenated technique combining HPLC with mass spectrometry.
7) **External Standard Method** – Quantification using calibration curve of standards.
8) **Internal Standard Method** – Quantification using a reference compound added to samples.
9) **Method Development** – Systematic optimization of chromatographic conditions.
10) **Resolution** – Measure of separation between two adjacent chromatographic peaks.

9.9. SELF-ASSESSMENT QUESTIONS:

A. Short Answer Questions:

1) What is the role of HPLC in organic chemistry?
2) Why is HPLC preferred for separation of complex organic mixtures?
3) What is the significance of retention time in compound identification?
4) Name any two methods used for quantitative estimation in HPLC.
5) What is the purpose of HPLC method development?

B. Descriptive / Essay Questions:

1) Discuss the applications of HPLC in the separation of organic compounds.
2) Explain the role of HPLC in identification of organic compounds.
3) Describe quantitative estimation of organic compounds by HPLC.
4) Discuss the applications of HPLC in pharmaceutical and natural product analysis.
5) Explain the systematic steps involved in HPLC method development.
6) Write a detailed note on the importance of HPLC in reaction monitoring and purity assessment.

9.10. REFERENCES (STANDARD ATT TEXT BOOKS):

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2) **Skoog, D.A., West, D.M., Holler, F.J., & Crouch, S.R.** *Fundamentals of Analytical Chemistry*, 9th Edition, Cengage Learning.
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Prof. M. Subba Rao

LESSON-10

BASIC PRINCIPLES, TECHNIQUES, AND INSTRUMENTATION OF GAS CHROMATOGRAPHY

10.0. OBJECTIVES:

After studying this lesson, the student will be able to:

1) Explain the **basic principles of gas chromatography** and the mechanism of separation.
2) Describe the **different types of gas chromatography techniques**, including GLC, GSC, capillary GC, and temperature-programmed GC.
3) Understand the **instrumentation of gas chromatography** and the function of each component.
4) Apply GC techniques for the **separation, identification, and quantitative analysis** of volatile and semi-volatile compounds.

STRUCTURE:

10.1 Basic Principles of Gas Chromatography

10.2 Different Types of Gas Chromatography Techniques

10.2.1. Gas-Liquid Chromatography (GLC)

10.2.2. Gas-Solid Chromatography (GSC)

10.2.3. Packed Column Gas Chromatography

10.2.4. Capillary (Open-Tubular) Gas Chromatography

10.2.5. Isothermal Gas Chromatography

10.2.6. Temperature-Programmed Gas Chromatography

10.2.7. Gas Chromatography–Mass Spectrometry (GC–MS)

10.2.8. Headspace Gas Chromatography

10.2.9. Conclusion

10.3. Instrumentation of Gas Chromatography

10.3.1. Carrier Gas Cylinder

10.3.2. Pressure Regulator and Gas Purification System

10.3.3. Flow Controller / Electronic Pressure Control (EPC)

10.3.4. Sample Injector (Injection Port)

10.3.5. Chromatographic Column

10.3.6. Column Oven

10.3.7. Detector

10.3.8. Amplifier and Data System

10.3.9. Recorder / Computer Output

10.3.10. Conclusion

10.4. Summary

10.5. Technical Terms

10.6. Self-Assessment Questions

10.7. References Textbooks

10.1. BASIC PRINCIPLES OF GAS CHROMATOGRAPHY:

Gas Chromatography (GC) is a powerful and widely used **analytical separation technique** employed for the separation, identification, and quantitative determination of **volatile and semi-volatile organic compounds**. The technique is based on the **differential distribution of sample components between a mobile gaseous phase and a stationary phase**.

In GC, the **mobile phase** is an inert carrier gas such as helium, nitrogen, or hydrogen, which continuously flows through a column. The **stationary phase** is either a **liquid coated on an inert solid support** or a **solid adsorbent**, depending on the type of GC employed. The sample, introduced in very small quantities, is rapidly vaporized and swept into the column by the carrier gas.

As the vaporized sample travels through the column, each component interacts differently with the stationary phase depending on its **boiling point, polarity, molecular weight, and vapour pressure**. Components that have weaker interactions or lower boiling points move faster and elute earlier, whereas those with stronger interactions remain longer in the column. Thus, separation occurs as a result of differences in **retention behavior** of individual components.

10.2. DIFFERENT TYPES OF GAS CHROMATOGRAPHY TECHNIQUES:

Gas chromatography comprises a family of analytical techniques designed for the **separation, identification, and quantitative analysis of volatile and semi-volatile compounds**. The techniques are classified based on the **nature of the stationary phase**, **column configuration**, and **mode of temperature control or detection**. Selection of an appropriate GC technique depends on the volatility, polarity, molecular size of analytes, and the complexity of the sample matrix.

10.2.1. Gas-Liquid Chromatography (GLC)

Gas–liquid chromatography is the **most important and widely used GC technique**. In GLC, the stationary phase is a **high-boiling, non-volatile liquid** immobilized as a thin film on an inert solid support (packed columns) or coated directly onto the inner wall of a capillary column.

10.2.1.1 Principle:

Separation occurs by **partitioning of analytes between the gaseous mobile phase and the liquid stationary phase**. Components with greater solubility in the stationary phase are retained longer, while more volatile components elute earlier.

10.2.1.2. Features and Applications:

GLC provides high efficiency, reproducibility, and excellent resolution. It is extensively used for the analysis of **hydrocarbons, alcohols, esters, fatty acids, essential oils, pesticides, pharmaceuticals, and petrochemical products**. Most modern GC analyses are based on GLC.

10.2.2. Gas-Solid Chromatography (GSC):

In gas–solid chromatography, the stationary phase consists of a **solid adsorbent** such as activated carbon, silica gel, alumina, or molecular sieves.

10.2.2.1. Principle:

Separation is based on **adsorption of analyte molecules on the solid surface**, followed by desorption back into the mobile gas phase.

10.2.2.2. Characteristics and Uses:

GSC is particularly useful for **permanent gases and low-molecular-weight hydrocarbons**. However, strong adsorption often causes **peak tailing and irreversible retention**, limiting its application mainly to gas analysis rather than complex organic mixtures.

10.2.3. Packed Column Gas Chromatography

Packed column GC uses **short columns of relatively large internal diameter** packed with solid particles coated with stationary phase.

10.2.3.1. Characteristics:

- Simple construction and low cost
- Higher sample capacity
- Lower efficiency and resolution compared to capillary columns

10.2.3.2. Applications:

Packed columns are still used in **routine industrial analysis, gas analysis, and preparative GC**, where high resolution is not critical.

10.2.4. Capillary (Open-Tubular) Gas Chromatography

Capillary GC employs **long, narrow fused-silica columns** with the stationary phase coated as a thin film on the inner surface.

10.2.4.1. Advantages:

- Very high separation efficiency
- Excellent resolution and sensitivity
- Reduced band broadening

10.2.4.2. Types of Capillary Columns:

- WCOT (Wall-Coated Open Tubular)
- SCOT (Support-Coated Open Tubular)
- PLOT (Porous Layer Open Tubular)

Capillary GC is the **preferred technique for modern GC analysis**, especially for complex mixtures and trace-level determination.

10.2.5. Isothermal Gas Chromatography:

In isothermal GC, the column temperature is kept **constant throughout the analysis**.

10.2.5.1. Suitability:

This technique is suitable for samples containing components with **similar boiling points**.

10.2.5.2. Limitation:

It is less effective for mixtures with a wide boiling-point range, as late-eluting compounds may show excessive retention.

10.2.6. Temperature-Programmed Gas Chromatography:

Temperature-programmed GC involves **gradual increase of oven temperature during the run**.

10.2.6.1. Advantages:

- Enables separation of compounds with **widely different volatilities**
- Improves peak shape for late-eluting components
- Reduces total analysis time
- Enhances resolution in complex mixtures

This technique is widely used in **environmental, pharmaceutical, and petrochemical analysis**.

10.2.7. Gas Chromatography–Mass Spectrometry (GC–MS)

GC–MS is a powerful **hyphenated technique** that combines the separation capability of GC with the identification power of mass spectrometry.

10.2.7.1. Significance:

It provides **molecular weight information, fragmentation patterns, and structural identification**, along with high sensitivity and selectivity.

10.2.7.2. Applications:

Forensic science, drug identification, environmental monitoring, metabolomics, and toxicology.

10.2.8. Headspace Gas Chromatography:

In headspace GC, only the **vapour phase in equilibrium with a solid or liquid sample** is introduced into the GC system.

10.2.8.1. Advantages:

- Eliminates interference from non-volatile matrix components
- Improves column and detector life

10.2.8.2. Applications:

Residual solvent analysis, food flavour analysis, pharmaceutical quality control, and forensic samples.

10.2.9. Conclusion:

Gas chromatography includes several techniques tailored to different analytical requirements. Among them, **gas-liquid chromatography using capillary columns** dominates modern GC due to its high efficiency and versatility. Advanced approaches such as **temperature-programmed GC, headspace GC, and GC-MS** greatly extend the analytical capability of gas chromatography, making it an indispensable technique in analytical chemistry.

10.3. INSTRUMENTATION OF GAS CHROMATOGRAPHY:

A gas chromatograph is an integrated analytical system designed for the **separation, detection, and quantitative analysis of volatile and semi-volatile compounds**. In this technique, analytes are transported by an inert carrier gas through a chromatographic column, where separation occurs based on differential interaction with the stationary phase. Each component of the instrument plays a crucial role in ensuring efficient vaporization, controlled transport, high-resolution separation, and sensitive detection.

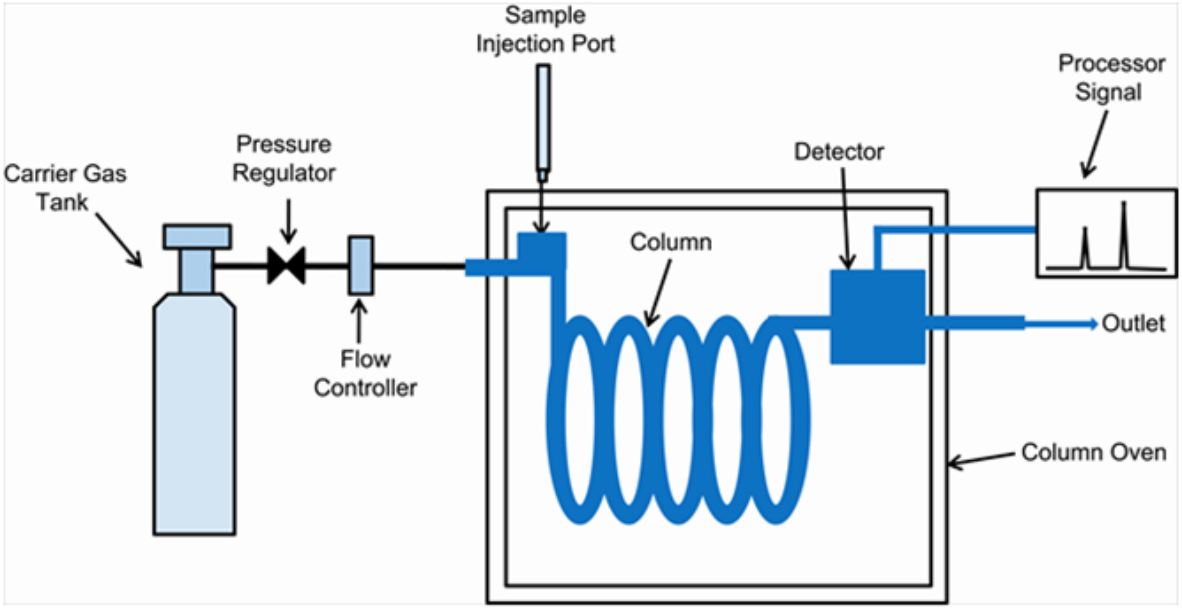

Figure 10.1: A Schematic Diagram of Instrument of Gas Chromatography

10.3.1. Carrier Gas Cylinder:

The carrier gas cylinder supplies the **mobile phase** for gas chromatography. The gas must be **chemically inert, dry, and oxygen-free** to prevent chemical reactions with analytes or degradation of the stationary phase. Common carrier gases include **helium, nitrogen, hydrogen, and argon**, selected based on separation efficiency, detector compatibility, safety, and cost.

10.3.2. Pressure Regulator and Gas Purification System:

The pressure regulator reduces the high pressure of the gas cylinder to a controlled working pressure suitable for chromatographic operation. The gas then passes through **purification traps** (moisture, oxygen, and hydrocarbon traps) to remove impurities. This ensures **stable baseline, reproducible retention times, and long column life**.

10.3.3. Flow Controller / Electronic Pressure Control (EPC):

The flow controller maintains a **constant carrier-gas flow rate or inlet pressure** to the column. Modern instruments use electronic pressure control for precise and programmable regulation. Accurate flow control is essential because variations directly affect **retention time, resolution, and efficiency**.

10.3.4. Sample Injector (Injection Port):

The injector introduces a **small and reproducible amount of sample** into the carrier-gas stream. The injector is heated so that the sample is **instantaneously vaporized** and mixed uniformly with the carrier gas. Injection is commonly performed through a **self-sealing septum** using a microsyringe. Depending on sample concentration, **split, splitless, or on-column injection** modes are employed.

10.3.5. Chromatographic Column

The column is the **heart of the gas chromatograph**, where actual separation takes place. It may be a **packed column** or a **capillary (open-tubular) column** coated with a suitable stationary phase. Separation occurs due to differences in **volatility and interaction of analytes with the stationary phase**, causing components to migrate at different rates.

10.3.6. Column Oven:

The column is housed inside a **thermostatically controlled oven**. The oven temperature can be maintained constant (isothermal operation) or increased gradually with time (temperature programming). Precise temperature control is crucial because retention time and selectivity are **highly temperature-dependent**. Temperature programming allows efficient separation of compounds with a wide boiling-point range in a single run.

10.3.7. Detector:

The detector senses the separated components as they elute from the column and converts their presence into an **electrical signal**. The detector is maintained at a temperature higher than the column outlet to prevent condensation. Common detectors include **Flame Ionization Detector (FID)**, **Thermal Conductivity Detector (TCD)**, and selective detectors such as **ECD, NPD, FPD**, and **GC–MS**. The choice of detector depends on sensitivity and selectivity requirements.

10.3.8. Amplifier and Data System:

The electrical signal generated by the detector is amplified and processed by a **computer-based data system**. The system records detector response as a function of time, generating a **chromatogram**. It also performs baseline correction, peak integration, and quantitative calculations.

10.3.9. Recorder / Computer Output:

The final output is displayed as a **chromatogram**, in which individual peaks correspond to different components of the mixture. **Retention time** is used for qualitative identification, while **peak area or peak height** is used for quantitative estimation.

10.3.10. Conclusion:

Thus, the gas chromatograph operates as an integrated system in which the **carrier gas transports the vaporized sample**, the **injector and column oven enable efficient separation**, the **detector senses eluting components**, and the **data system converts signals into a meaningful chromatogram** for qualitative and quantitative analysis.

10.4. SUMMARY:

1) Gas chromatography is a widely used analytical technique for **separating volatile and semi-volatile organic compounds** based on differential retention.

2) Separation occurs due to differences in **boiling point, polarity, vapour pressure, and interaction with the stationary phase**.
3) GC techniques include **gas-liquid chromatography, gas-solid chromatography, packed and capillary column GC, isothermal and temperature-programmed GC**, and advanced techniques such as **GC–MS and headspace GC**.
4) The GC instrument consists of a **carrier gas system, injector, column oven, column, detector, and data system**, all working together to produce a chromatogram.
5) GC is an essential tool in **environmental, pharmaceutical, petrochemical, forensic, and industrial analysis**.

10.5. TECHNICAL TERMS:

1) **Gas Chromatography (GC)** – A chromatographic technique using a gas as the mobile phase.
2) **Carrier Gas** – An inert gas that transports analytes through the GC column.
3) **Stationary Phase** – The phase that selectively retains analytes during separation.
4) **Gas–Liquid Chromatography (GLC)** – GC in which the stationary phase is a liquid.
5) **Gas–Solid Chromatography (GSC)** – GC using a solid adsorbent as stationary phase.
6) **Packed Column** – A GC column filled with solid particles coated with stationary phase.
7) **Capillary Column** – A narrow open-tubular column with stationary phase on the inner wall.
8) **Temperature Programming** – Gradual increase of oven temperature during analysis.
9) **Isothermal Operation** – GC operation at constant column temperature.
10) **Detector** – Device that converts eluting analytes into an electrical signal.
11) **Flame Ionization Detector (FID)** – Detector measuring ions formed during combustion.
12) **Thermal Conductivity Detector (TCD)** – Detector measuring changes in thermal conductivity.
13) **Retention Time (t)** – Time taken by a compound to elute from the column.
14) **Chromatogram** – Plot of detector response versus time.
15) **GC–MS** – Hyphenated technique combining GC with mass spectrometry.

10.6. SELF-ASSESSMENT QUESTIONS:

A. Short Answer Questions

1) Define gas chromatography.
2) What is the role of the carrier gas in GC?
3) Differentiate between GLC and GSC.
4) What is temperature-programmed gas chromatography?
5) State two advantages of capillary GC.
6) Name any two GC detectors.
7) What is headspace gas chromatography?

B. Descriptive / Essay Questions

1) Explain the **basic principles of gas chromatography**.
2) Discuss the **different types of gas chromatography techniques**.
3) Describe **gas-liquid chromatography and its applications**.
4) Write a detailed note on the **instrumentation of gas chromatography with a labeled diagram**.
5) Explain the **role of column oven and temperature programming in GC**.
6) Discuss the **advantages and applications of GC-MS**.

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1) **Skoog, D.A., Holler, F.J., & Crouch, S.R.** *Principles of Instrumental Analysis*, 6th Edition, Cengage Learning.
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Dr. P. Bharath

LESSON-11

SELECTION OF COLUMNS, CARRIER GASES, AND DETECTORS IN GAS CHROMATOGRAPHY

11.0. OBJECTIVES:

After studying this lesson, the student will be able to:

1) Understand the **importance and selection of GC columns** for efficient chromatographic separation.
2) Differentiate between **packed columns and capillary (open-tubular) columns** used in gas chromatography.
3) Explain the **role and selection of carrier gases** and compare helium, nitrogen, and hydrogen.
4) Describe the **principles, advantages, and limitations of common GC detectors** such as FID, TCD, and ECD.
5) Select suitable **columns, carrier gases, and detectors** for specific analytical applications.

STRUCTURE:

11.1 Selection of Column

11.1.1. Importance of GC Column

11.1.2. Classification of GC Columns

11.1.3. Factors Governing Column Selection

11.1.4. Role of Stationary Phase Polarity

11.1.5. Effect of Column Dimensions

11.2 Selection of Carrier Gases

11.2.1. Role of Carrier Gas in GC

11.2.2. Characteristics of an Ideal Carrier Gas

11.2.3. Commonly Used Carrier Gases

11.2.4. Factors Governing the Choice of Carrier Gas

11.3 Detectors Used in Gas Chromatography

11.3.1. Role of Detectors in GC

11.3.2. Flame Ionization Detector (FID)

11.3.3. Thermal Conductivity Detector (TCD)

11.3.4. Electron Capture Detector (ECD)

11.4 Summary

11.5 Technical Terms

11.6 Self-Assessment Questions

11.7 References Textbooks

11.1. SELECTION OF COLUMN:

11.1.1. Importance of GC Column:

The column is the **most critical component of a gas chromatographic system**, as the actual separation of sample components takes place within it. The efficiency of separation, resolution of peaks, sensitivity, and overall analytical performance of gas chromatography are largely governed by the **type of column and stationary phase employed**. An appropriate choice of column ensures sharp, symmetrical peaks and reliable qualitative and quantitative analysis.

11.1.2. Classification of GC Columns:

Based on construction and mode of operation, GC columns are broadly classified into:

a) Packed Columns

b) Capillary (Open Tubular) Columns

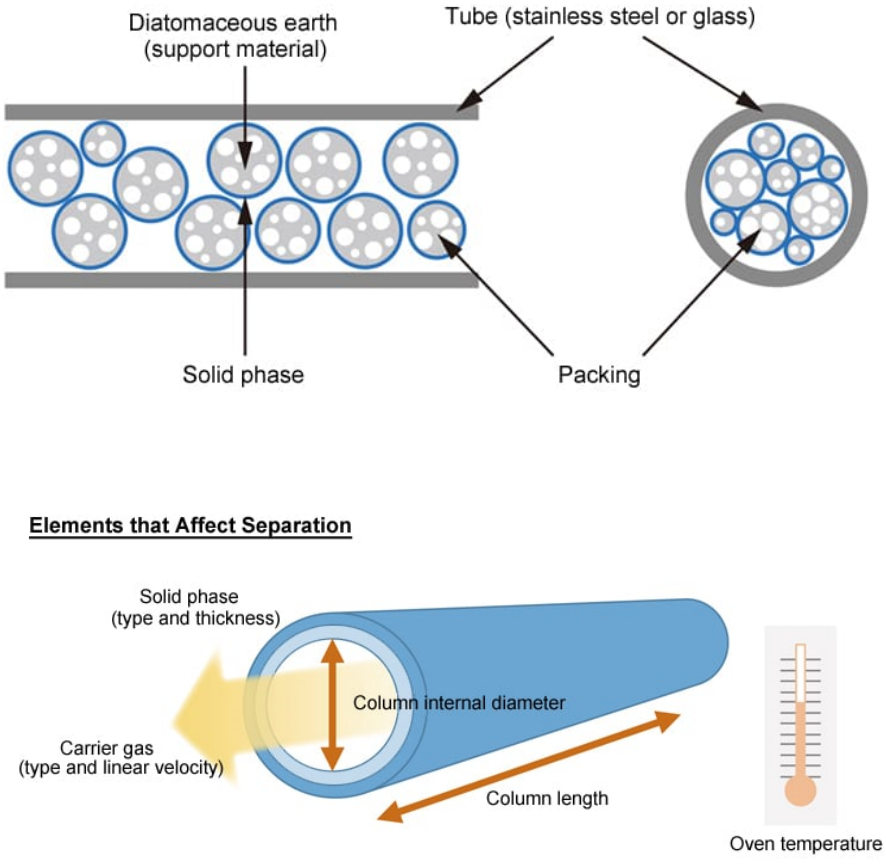

Figure 11.1: Types of Gas Chromatography Columns

a) Packed Columns:

Packed columns are **short and relatively wide tubes** made of stainless steel or glass, filled with **solid support particles coated with a liquid stationary phase**. These columns are **simple in design, mechanically robust, and economical**, making them suitable for routine analyses.

Due to their larger internal diameter, packed columns permit **higher sample loading**, which is advantageous in preparative or industrial applications. However, they generally exhibit **lower efficiency, broader peaks, and poorer resolution** compared to capillary columns. As a result, packed columns are mainly used for **simple mixtures and gas analysis**, where extremely high resolution is not required.

b) Capillary (Open Tubular) Columns:

Capillary columns are **long, narrow tubes**, usually fabricated from **fused silica**, with the stationary phase coated as a **thin uniform film on the inner wall**. The absence of packing material significantly reduces band broadening, resulting in **very high separation efficiency, superior resolution, and enhanced sensitivity**.

Capillary columns are particularly suitable for the **analysis of complex mixtures and trace-level compounds**, and they dominate modern gas chromatographic practice. Their excellent performance makes them indispensable in environmental, pharmaceutical, forensic, and petrochemical analyses.

11.1.3. Factors Governing Column Selection:

The selection of a suitable GC column depends on several factors such as:

a) Polarity of analytes

b) Boiling point range

c) Thermal stability of compounds

d) Required resolution and analysis time

11.1.4. Role of Stationary Phase Polarity:

The polarity of the stationary phase plays a decisive role in chromatographic separation. In general, optimal separation is achieved when the **polarity of the stationary phase closely matches that of the analyte**, following the chromatographic principle **"like dissolves like."** This enhances interactions between analyte molecules and the stationary phase, resulting in improved retention and resolution.

11.1.5. Effect of Column Dimensions:

Column parameters such as **length, internal diameter, and stationary-phase film thickness** significantly influence chromatographic performance. Longer columns and smaller internal diameters provide higher resolution but increase analysis time and backpressure. Therefore, column selection involves a **practical compromise between efficiency, resolution and speed of analysis**.

11.2. SELECTION OF CARRIER GASES:

11.2.1 Role of Carrier Gas in GC

In gas chromatography, the carrier gas functions as the **mobile phase**, transporting the vaporized sample through the column. The **nature, purity, and flow rate** of the carrier gas strongly influence separation efficiency, retention time, detector response, and overall reproducibility. Hence, selecting an appropriate carrier gas is a crucial step in GC method development.

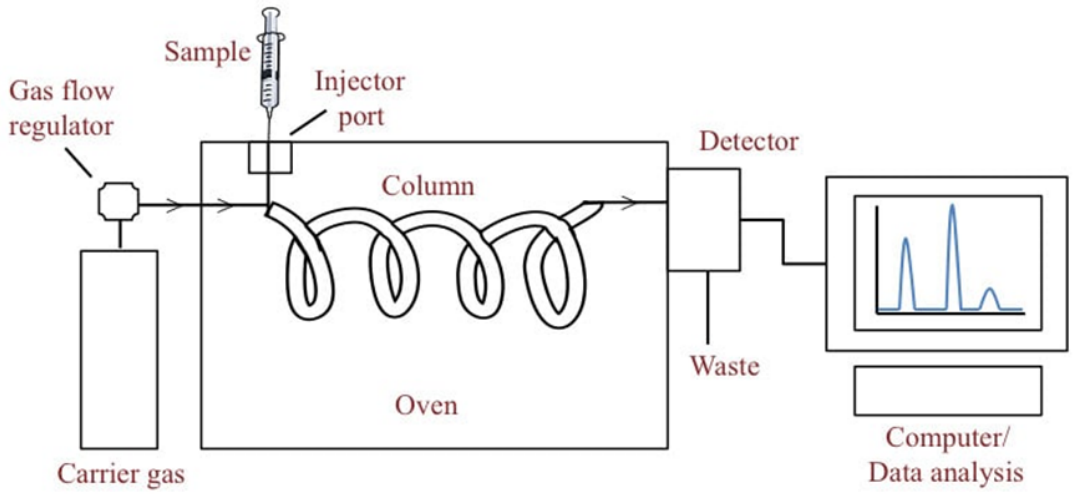

Figure 11.2: Carrier Gas Supply and Flow Control System

11.2.2 Characteristics of an Ideal Carrier Gas:

An ideal carrier gas should possess the following properties:

i) **Chemical inertness**, so that it does not react with the analyte or stationary phase
ii) **High purity**, to prevent baseline noise and contamination
iii) **Good flow characteristics**, ensuring uniform and reproducible separations
iv) **Compatibility with the detector** used in the analysis

11.2.3 Commonly Used Carrier Gases:

Helium:

Helium is one of the most commonly used carrier gases in gas chromatography. It is **chemically inert** and provides **good separation efficiency over a wide range of flow rates**. Helium is particularly suitable for use with **capillary columns** and is compatible with most detectors, including FID and MS. However, its main limitation is its **high cost and limited availability**.

Nitrogen:

Nitrogen is an **economical and inert** carrier gas and is widely used in routine analyses. It offers **high efficiency at low flow rates**, making it suitable for certain packed column applications. However, at higher flow rates, nitrogen shows **reduced efficiency and broader peaks**, leading to longer analysis times when high resolution is required.

Hydrogen:

Hydrogen is an excellent carrier gas due to its **high diffusivity and low viscosity**, which result in **fast separations and high column efficiency**. It is particularly advantageous for high-speed analyses and long capillary columns. Despite these benefits, hydrogen requires **strict safety precautions** because of its **flammable and explosive nature**, limiting its use in some laboratories.

Table: Brief comparison of performance capability of helium, hydrogen, and nitrogen

Parameter	Helium	Hydrogen	Nitrogen
Safety	Safe	Caution	Safe
Source	Cylinder	Cylinder or generator	Cylinder or generator
Cost	Expensive	Cost effective	Cost effective
Supply	Concern	Not applicable	Not applicable
Column choices	Wide to narrow bore	Narrow bore	Narrow bore
Inertness	Inert	Highly reactive	Inert
BFB/DFTPP	Passes	Passes	Passes

11.2.4 Factors Governing the Choice of Carrier Gas:

The selection of a carrier gas depends on several analytical and practical considerations, including:

i) Type of column (packed or capillary)
ii) Type of detector employed
iii) Required resolution and analysis speed
iv) Safety and laboratory infrastructure
v) Cost and availability of gas

11.3. DETECTORS USED IN GAS CHROMATOGRAPHY:

11.3.1 Role of Detectors in GC:

In gas chromatography, detectors play a crucial role in the **identification and quantitative determination of separated components**. As the analytes elute from the chromatographic column, the detector senses their presence and converts it into an **electrical signal**. The magnitude of this signal is related to the concentration of the analyte, allowing both **qualitative analysis (via retention time)** and **quantitative analysis (via peak area or height)**. The choice of detector depends on factors such as **sensitivity, selectivity, linear range, and the nature of analytes**.

11.3.2. Flame Ionization Detector (FID):

The **Flame Ionization Detector (FID)** is the most widely used detector in gas chromatography, particularly for the analysis of **organic compounds**. In this detector, the column effluent is mixed with hydrogen and air and burned in a small flame. Organic compounds produce ions and electrons during combustion, which generate an **ion current** when collected at electrodes.

The detector response is approximately proportional to the **number of carbon atoms** present in the analyte, making FID highly suitable for hydrocarbons and most organic substances.

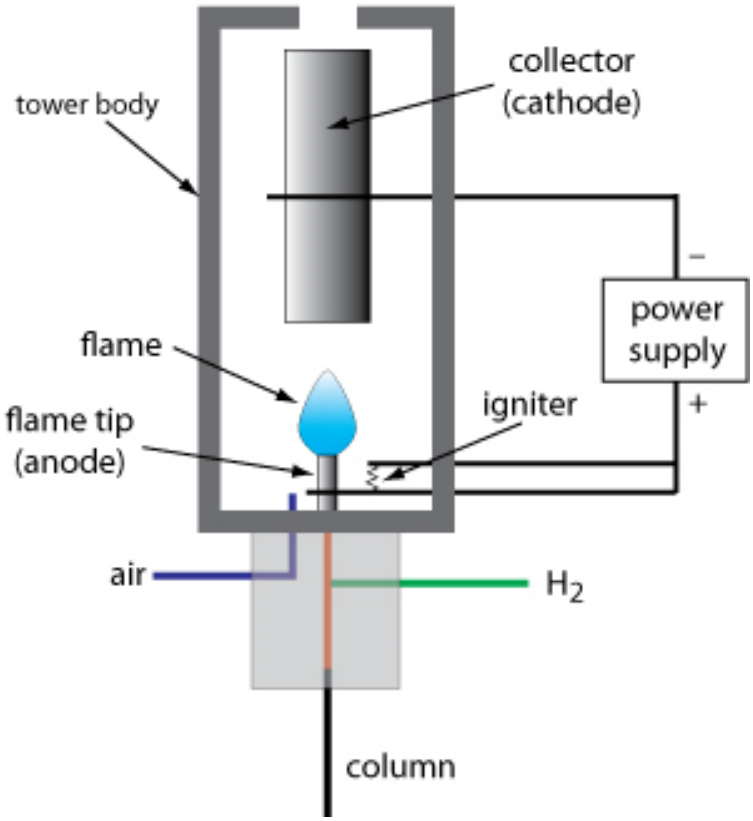

Figure 11.3: Flame Ionization Detector (FID)

Advantages of FID:

- Very **high sensitivity**
- **Wide linear dynamic range**
- Excellent stability and reproducibility

Limitations of FID:

- Destructive detector
- Poor response to inorganic gases such as CO_2, H_2O, and noble gases

11.3.3. Thermal Conductivity Detector (TCD):

The **Thermal Conductivity Detector (TCD)** is a **universal and non-destructive detector** that operates by measuring changes in the **thermal conductivity of the gas stream**. It consists of heated filaments arranged in a Wheatstone bridge configuration. When analyte molecules elute from the column, they alter the thermal conductivity of the carrier gas, causing a change in filament temperature and resistance, which is detected as a signal.

Because it responds to almost all compounds, the TCD is particularly useful for **permanent gases and inorganic compounds**.

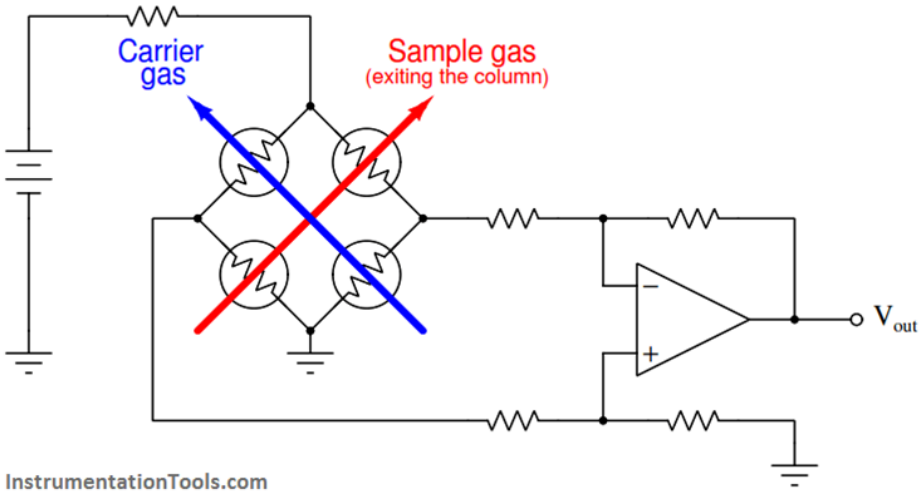

Figure 11.4: Thermal Conductivity Detector (TCD)

Advantages of TCD:

- Universal response
- Non-destructive
- Suitable for inorganic and permanent gases

Limitations of TCD:

- Lower sensitivity compared to FID
- Requires high-purity carrier gases

11.3.4. Electron Capture Detector (ECD):

The **Electron Capture Detector (ECD)** is a highly sensitive and selective detector, especially for compounds containing **electronegative functional groups**. It operates using a **radioactive β-source** (commonly Ni-63) that produces a steady stream of electrons. When compounds with high electron affinity (such as halogenated compounds) enter the detector, they capture electrons, resulting in a **decrease in current**, which is measured as the detector response.

ECD is extensively used in **environmental analysis**, particularly for the detection of **pesticides, herbicides, and halogenated hydrocarbons**.

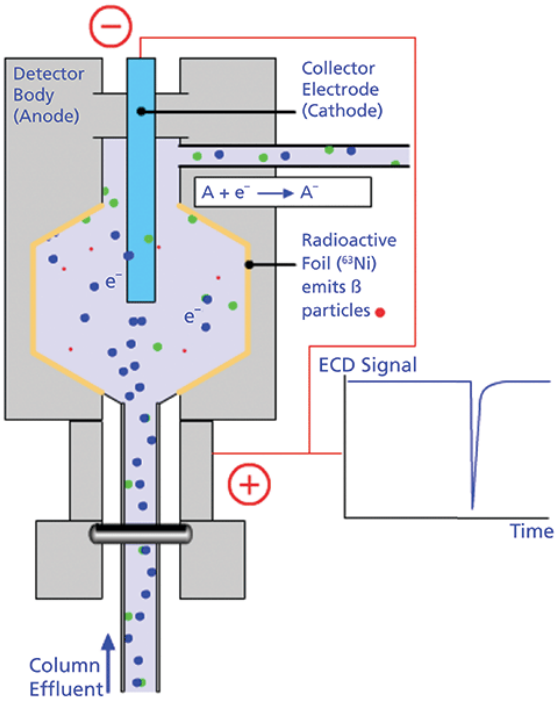

Figure 11.5: Electron Capture Detector (ECD)

Advantages of ECD:

- Extremely high sensitivity
- Highly selective for halogen-containing compounds

Limitations of ECD:

- Limited applicability to specific compounds
- Requires careful control of operating conditions

11.4. SUMMARY:

1) The **GC column** is the most critical component of a gas chromatographic system, directly influencing resolution, efficiency, and sensitivity.
2) GC columns are classified into **packed columns** and **capillary (open-tubular) columns**, with capillary columns offering superior efficiency and resolution.
3) Column selection depends on **analyte polarity, boiling-point range, thermal stability, and required resolution**.
4) The **polarity of the stationary phase** plays a key role in separation, following the principle *"like dissolves like."*
5) The **carrier gas** acts as the mobile phase and significantly affects retention time, separation efficiency, and detector performance.
6) **Helium, nitrogen, and hydrogen** are the most commonly used carrier gases, each having specific advantages and limitations.

7) **Detectors** convert the presence of eluting analytes into electrical signals for qualitative and quantitative analysis.
8) **FID** is widely used for organic compounds, **TCD** is universal and non-destructive, and **ECD** is highly selective for halogenated compounds.

11.5. TECHNICAL TERMS:

1) **Gas Chromatography (GC)** – A chromatographic technique using a gas as the mobile phase.
2) **Packed Column** – A GC column packed with solid particles coated with stationary phase.
3) **Capillary Column** – A narrow open-tubular column with stationary phase coated on the inner wall.
4) **Stationary Phase** – The phase responsible for retention and separation of analytes.
5) **Carrier Gas** – Inert gas that transports analytes through the GC column.
6) **Helium** – Common carrier gas providing good efficiency and inertness.
7) **Nitrogen** – Economical carrier gas with good efficiency at low flow rates.
8) **Hydrogen** – Fast and efficient carrier gas requiring safety precautions.
9) **Flame Ionization Detector (FID)** – Detector measuring ions formed during combustion of organic compounds.
10) **Thermal Conductivity Detector (TCD)** – Universal detector based on changes in thermal conductivity.
11) **Electron Capture Detector (ECD)** – Highly sensitive detector for electronegative compounds.
12) **Retention Time (t)** – Time taken by an analyte to elute from the column.
13) **Chromatogram** – Plot of detector response versus time.
14) **Resolution** – Measure of separation between adjacent chromatographic peaks.

11.6 SELF-ASSESSMENT QUESTIONS

A. Short Answer Questions

1) Why is the column calling the heart of a gas chromatograph?
2) Distinguish between packed and capillary columns.
3) State the characteristics of an ideal carrier gas.
4) Why is helium preferred in capillary GC?
5) What is the principle of the flame ionization detector?
6) Mention two advantages of the thermal conductivity detector.
7) Name applications of the electron capture detector.

B. Descriptive / Essay Questions

1) Discuss the **selection and classification of GC columns**.
2) Explain the **factors governing column selection** in gas chromatography.
3) Describe the **role and selection of carrier gases** with a comparison of helium, nitrogen, and hydrogen.
4) Explain the **principle, advantages, and limitations of FID**.
5) Write notes on **TCD and ECD detectors** with suitable applications.
6) Discuss the **importance of selecting suitable columns, carrier gases, and detectors** for efficient GC analysis.

11.7. REFERENCES (STANDARD ANALYTICAL CHEMISTRY TEXTBOOKS)

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LESSON-12

RETENTION TIME AND APPLICATIONS OF GAS CHROMATOGRAPHY

12.0. OBJECTIVES:

After completing this lesson, the student will be able to:

1) Define **retention time, dead time, and adjusted retention time** in gas chromatography.
2) Explain the **system-dependent nature of retention time** and factors affecting it.
3) Understand the **role of retention time in qualitative identification** of organic compounds.
4) Describe the **use of GC in separation, identification, and quantitative analysis** of organic compounds.
5) Apply GC concepts to **reaction monitoring, impurity profiling, and multicomponent analysis**.

STRUCTURE:

12.1 Retention Time in Gas Chromatography

12.2 Definition of Retention Time (t)

12.3 Factors Affecting Retention Time

12.3.1. Column Temperature

12.3.2. Nature of the Stationary Phase

12.3.3. Carrier Gas Flow Rate

12.3.4. Volatility of the Analyte

12.4 Use of Retention Time in Quantitative Gas Chromatography

12.5 Dead Time (t_m) and Adjusted Retention Time (t')

12.6 Applications of Gas Chromatography in the Separation, Consideration and Quantitative Analysis of Organic Compounds

12.6.1. Separation of Organic Compounds

12.6.2. Identification of Organic Compounds

12.6.3. Quantitative Analysis of Organic Compounds

12.6.4. Integrated Use: Separation, Identification, and Quantification

12.7 Summary

12.8 Technical Terms

12.9 Self-Assessment Questions

12.10 Reference Books

12.1. RETENTION TIME IN GAS CHROMATOGRAPHY:

Retention time is a fundamental concept in gas chromatography and forms the basis for **qualitative identification** as well as **reliable quantitative analysis**. A clear understanding of its definition, significance, limitations, and related parameters is essential for chromatographic method development, optimization, and interpretation of results.

12.2. DEFINITION OF RETENTION TIME (t):

Retention time (t_r) is defined as the **time interval between the injection of a sample into the chromatographic system and the appearance of the maximum (apex) of the corresponding peak at the detector**. It is measured along the time axis of the chromatogram from the point of injection (time zero) to the peak maximum.

Under strictly controlled operating conditions-such as column type and dimensions, stationary phase chemistry, oven temperature (isothermal or programmed), carrier gas identity, and flow rate-each compound exhibits a **characteristic and reproducible retention time**. Retention time reflects the duration for which an analyte resides in the column and is governed by its distribution between the mobile gas phase and the stationary phase. Strongly retained analytes exhibit larger t_r values, whereas weakly retained analytes elute earlier with smaller t_r values.

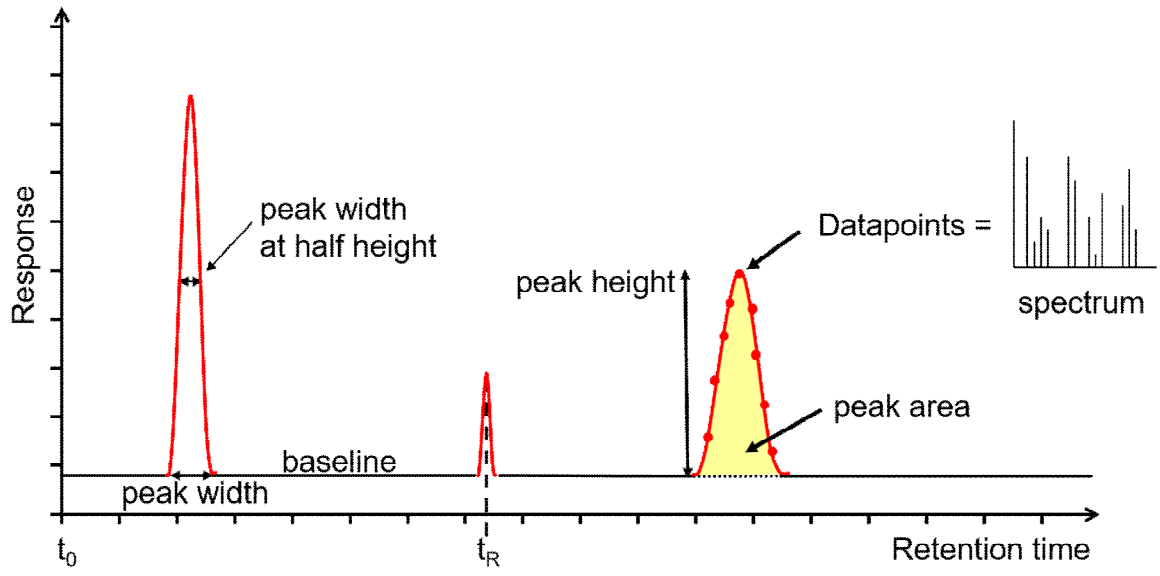


Figure 12.1: Determination of Retention Time (t) in Gas Chromatography

Retention Time and Qualitative Identification:

In gas chromatography, retention time is primarily used for **qualitative identification of compounds**. When chromatographic conditions are held constant, a given compound consistently elutes at the same retention time within acceptable experimental tolerance. An unknown component can therefore be tentatively identified by comparing its retention time with that of a known reference standard analyzed under identical conditions.

However, **retention time matching alone does not conclusively establish identity**. Different compounds may occasionally exhibit very similar or overlapping retention times,

particularly on non-selective stationary phases or under non-optimized conditions. Consequently, retention time comparison provides **presumptive or screening-level identification**. Definitive identification often requires additional evidence from selective detectors or hyphenated techniques such as GC-MS, which provide molecular weight and structural information.

Retention Time as a System-Dependent Parameter:

Retention time is **not an intrinsic physical constant of a compound**; rather, it is a **system-dependent parameter**. It is strongly influenced by chromatographic operating conditions, including column dimensions, stationary phase composition and film thickness, carrier gas type and flow rate, oven temperature profile, injection mode, and instrument design.

As a result, retention time values are meaningful only when experimental conditions are carefully **controlled, standardized, and documented**. During method transfer or inter-laboratory comparisons, even small variations in conditions can lead to shifts in retention time. For more robust comparison, relative parameters such as **adjusted retention time, capacity factor, or retention indices** are sometimes preferred.

12.3. FACTORS AFFECTING RETENTION TIME:

12.3.1. Column Temperature:

Column temperature has a pronounced and often dominant influence on retention time. Increasing the oven temperature increases the **vapour pressure of analytes**, reduces their partitioning into the stationary phase, and consequently **shortens retention times**. Lower temperatures increase retention and may improve resolution of closely eluting components, but at the cost of longer analysis times.

Temperature is the most powerful variable for controlling retention and forms the basis of **temperature-programmed gas chromatography**, which enables efficient separation of components with widely differing volatilities.

12.3.2. Nature of the Stationary Phase:

Retention time depends on the **strength and selectivity of interactions between analyte molecules and the stationary phase**. Polar analytes are generally retained more strongly on polar stationary phases, while non-polar analytes show greater retention on non-polar phases, consistent with the principle **"like interacts with like."** Even small changes in stationary-phase polarity, functional groups, or film thickness can significantly influence retention behaviour and elution order.

12.3.3. Carrier Gas Flow Rate:

Retention time is inversely related to carrier gas flow rate. Increasing the flow rate decreases t_r, whereas decreasing the flow rate increases t_r. Although higher flow rates reduce analysis time, excessively high or low flow rates can move the system away from optimum efficiency, leading to broader peaks and reduced resolution. Therefore, the flow rate must be carefully optimized to balance **speed, efficiency, and detector compatibility**.

12.3.4. Volatility of the Analyte:

Volatility is a major determinant of retention time. More volatile compounds with lower boiling points spend more time in the gas phase, interact less with the stationary phase, and elute earlier. Less volatile compounds remain longer in the stationary phase and elute later. This effect is especially pronounced on non-polar stationary phases, where separation is largely governed by differences in boiling point.

12.4. USE OF RETENTION TIME IN QUANTITATIVE GAS CHROMATOGRAPHY:

Retention time itself is **not used directly for quantification**. Instead, it serves to **locate and assign peaks** to specific analytes on the chromatogram. Quantitative information is obtained from **peak area (most commonly) or peak height**, which are proportional to the amount or concentration of analyte reaching the detector under linear response conditions.

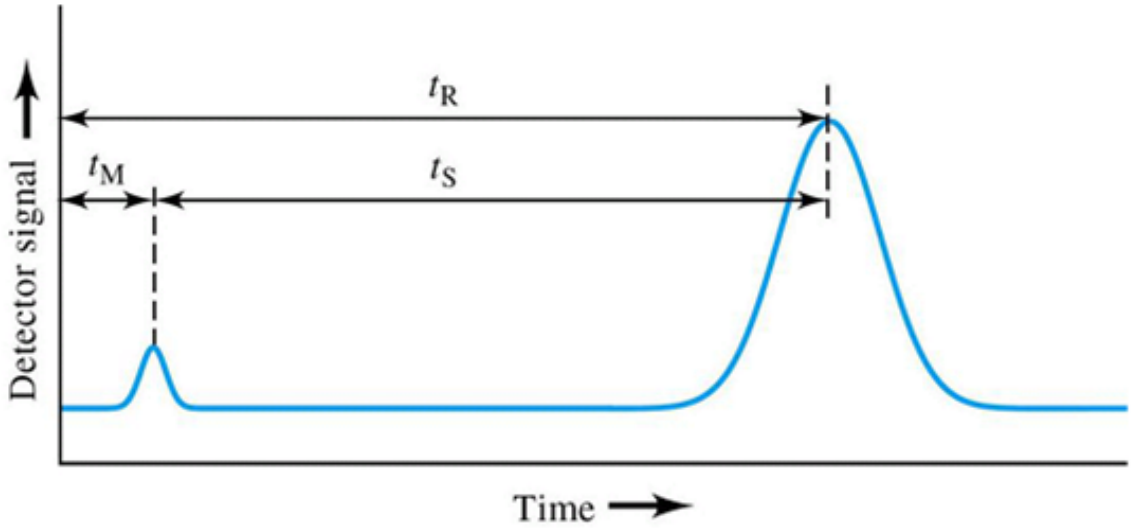
Nevertheless, accurate and reproducible retention times are essential for reliable quantification, as incorrect peak assignment leads to erroneous concentration values. Stable retention times ensure correct matching with calibration standards and facilitate automated peak integration in routine analyses.

12.5. DEAD TIME (T_m) AND ADJUSTED RETENTION TIME (t′)

12.5.1. Dead Time (t_m) / Hold-Up Time:

Dead time (t_m), also called hold-up time, is the **time taken by an unretained component-or effectively by the carrier gas-to pass through the column and reach the detector**. It represents the minimum possible retention time in a chromatographic system and corresponds to the portion of the analyte's journey spent entirely in the mobile phase.

Experimentally, t_m is determined by injecting a non-retained marker such as methane or air and measuring its peak time.



Relationship between Retention Time and Dead Time,

$$t_r = t_m + t_s$$

where,

t = retention time; $\mathbf{t_m}$ = dead time (void time)

$\mathbf{t_s}$ = time duration for which the analyte is retained in the stationary phase

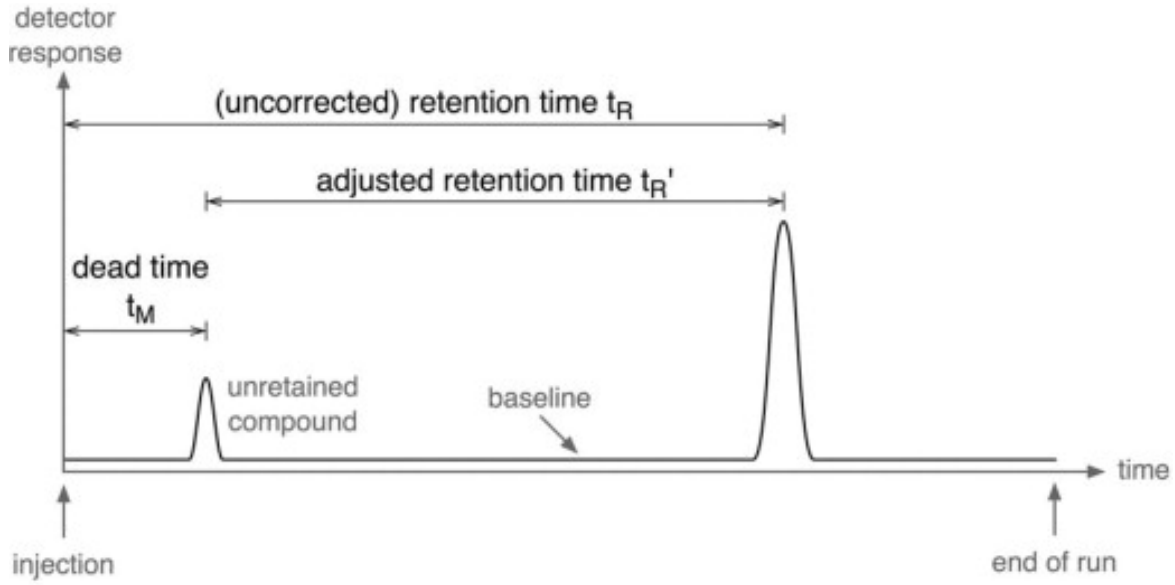
12.5.2 Adjusted Retention Time (t′):

Adjusted retention time (t'_r) corrects the observed retention time for the contribution of dead time and represents the portion of retention due solely to interaction with the stationary phase. It is defined as:

$$t\prime_r = t_r - t_m$$

Where, t_r = observed retention time

t_m = dead (hold-up) time



Adjusted retention time provides a **more meaningful measure of chromatographic retention** and is useful for comparing analyte behaviour and for calculating other chromatographic parameters such as the capacity factor.

12.6. APPLICATIONS OF GAS CHROMATOGRAPHY IN THE SEPARATION, IDENTIFICATION, AND QUANTITATIVE ANALYSIS OF ORGANIC COMPOUNDS:

Gas chromatography (GC) is one of the most powerful and versatile analytical techniques for the analysis of **volatile and semi-volatile organic compounds** encountered in organic synthesis, pharmaceutical formulations, environmental samples, and biological matrices. At the postgraduate level, GC should be viewed not merely as an instrumental method, but as an **integrated analytical approach** that combines high-resolution separation, reliable qualitative identification, and accurate quantitative determination.

12.6.1. Separation of Organic Compounds:

12.6.1.1. Separation of Reaction Mixtures and Synthetic Intermediates:

Gas chromatography is routinely employed to resolve complex organic reaction mixtures into their individual components. Following a synthetic transformation, the crude mixture may contain unreacted starting materials, the desired product, side products, and residual solvents. GC enables:

- Efficient separation of components based on differences in volatility and interaction with the stationary phase.
- Rapid monitoring of reaction progress by comparing relative peak areas of reactants and products in samples withdrawn at different times.
- Optimization of reaction parameters such as temperature, time, catalyst, and solvent.
- Informed decisions regarding downstream purification methods, including distillation, extraction, or chromatographic isolation.

For low-boiling intermediates such as alkyl halides, esters, aldehydes, ketones, nitriles, and small amides, **capillary GC coupled with flame ionization detection (FID)** provides excellent resolution and sensitivity within short analysis times.

12.6.1.2. Separation of Isomeric Organic Compounds:

Isomeric compounds commonly arise in organic synthesis and natural product chemistry, and their separation is often analytically challenging. GC offers powerful resolving capability for:

- **Positional isomers** (e.g., ortho-, meta-, and para-substituted aromatics), which differ subtly in polarity and boiling point.
- **Geometric isomers** (cis/trans or E/Z alkenes), where differences in molecular shape and dipole moment affect retention.
- **Diastereomers**, which may be separated on conventional stationary phases due to differing physicochemical properties.
- **Enantiomers**, using specialized **chiral capillary columns** (e.g., cyclodextrin-based phases), a critical requirement in chiral drug analysis and asymmetric synthesis.

Such separations allow chemists to evaluate **isomeric purity**, stereoselectivity, and reaction pathways with high confidence.

12.6.1.3. Separation in Multicomponent Organic Matrices:

In applied and industrial contexts, GC is indispensable for separating highly complex organic mixtures, including:

- Hydrocarbon fractions in petrochemical products such as gasoline, diesel, and lubricants.
- Aroma and flavour constituents (terpenes, esters, aldehydes) in foods, beverages, essential oils, and fragrances.
- Environmental pollutants such as volatile organic compounds (VOCs), halogenated solvents, and pesticide residues.

Temperature-programmed capillary GC, and in advanced applications **multidimensional GC or GC×GC**, provide high peak capacity and allow resolution of dozens to hundreds of components within a single chromatogram.

12.6.2 Identification of Organic Compounds:

12.6.2.1. Retention Time Comparison:

Retention time (t_r) is the primary parameter used for routine qualitative identification in GC. When chromatographic conditions are carefully controlled—same column and stationary phase, temperature programme, carrier gas, flow rate, and injection mode—each compound exhibits a characteristic and reproducible retention time.

An unknown component can therefore be tentatively identified by comparing its t_r with that of an authentic reference standard analyzed under identical conditions. To improve reliability, **relative retention values** or **retention indices** (e.g., Kovats index) are often employed by referencing analyte retention to a homologous series such as n-alkanes. This approach minimizes the effect of small variations in operating conditions and enables inter-laboratory comparison.

12.6.2.2. Detector Selectivity and Class Identification:

Retention time data are frequently complemented by the selectivity of the detector used:

- **Flame Ionization Detector (FID):** A near-universal detector for organic compounds, providing a response approximately proportional to the number of carbon atoms.
- **Electron Capture Detector (ECD):** Highly sensitive toward electronegative compounds, particularly halogenated and nitro-containing organics.
- **Nitrogen–Phosphorus Detector (NPD):** Selective for nitrogen- and phosphorus-containing compounds, widely used in pharmaceutical and agrochemical analysis.

Such selective responses help confirm the **chemical class** of an eluting compound and strengthen qualitative identification beyond retention time alone.

12.6.2.3. GC-MS for Structural Identification:

Coupling gas chromatography with mass spectrometry (GC–MS) provides a powerful platform for definitive identification:

- The GC column temporally separates components, reducing spectral overlap.
- The mass spectrometer provides molecular-ion and fragment-ion information, enabling determination of molecular weight and structural features.
- Acquired spectra can be matched against reference libraries, allowing high-confidence identification even at trace levels.

GC–MS is extensively used to confirm product identity in organic synthesis, characterize unknown by-products, and perform confirmatory analyses in forensic, environmental, and biomedical applications.

12.6.3. QUANTITATIVE ANALYSIS OF ORGANIC COMPOUNDS:

12.6.3.1. Relationship Between Peak Area and Analyte Amount:

The basis of quantitative GC lies in the **direct proportionality between detector response and the amount of analyte** reaching the detector. Each separated component produces a chromatographic peak, and the **peak area** (more commonly than peak height) is proportional to analyte mass or concentration within the detector's linear range.

Accurate quantification requires baseline-resolved peaks with minimal tailing or overlap, ensuring that each integrated peak corresponds to a single analyte.

12.6.3.2. Calibration Approaches:

External Standard Method:

- Standard solutions of known analyte concentration are analyzed under identical conditions as the sample.
- A calibration curve of peak area versus concentration is constructed.
- Sample concentrations are obtained by interpolation.
- Widely used in routine quality control, solvent purity testing, and simple formulations.

Internal Standard Method:

- A known amount of an internal standard—chemically stable, absent from the sample, and well resolved-is added to both standards and samples.
- Quantification is based on the ratio of analyte peak area to internal standard peak area.
- This method compensates for variations in injection volume, sample loss, and short-term detector drift, and is particularly valuable in complex matrices.

Typical Quantitative Applications:

- **Pharmaceutical analysis:** Assay of active pharmaceutical ingredients and determination of impurities and residual solvents.
- **Reaction monitoring:** Calculation of yields, selectivity, and by-product formation in organic synthesis.
- **Environmental analysis:** Measurement of VOCs and hazardous organic pollutants in air, water, and soil.
- **Food and flavour chemistry:** Quantification of aroma compounds, off-flavours, and regulated contaminants.

GC routinely achieves detection limits at ppm to ppb levels, meeting regulatory and quality-assurance requirements.

12.6.4. Integrated Use: Separation, Identification, and Quantification:

In practice, gas chromatography integrates three essential analytical functions:

- **Separation**, achieved through appropriate choice of column chemistry and temperature programming.
- **Identification**, based on retention time comparison, detector selectivity, and, when required, mass-spectral information.
- **Quantification**, using calibrated detector responses to determine analyte concentrations with accuracy and precision.

12.7. SUMMARY:

1) Retention time is a fundamental chromatographic parameter used mainly for **qualitative identification** in GC.
2) It is **system-dependent** and influenced by column temperature, stationary phase, carrier-gas flow rate, and analyte volatility.
3) **Dead time (t_m)** represents mobile-phase transit time, while **adjusted retention time (t')** reflects true interaction with the stationary phase.
4) GC enables efficient **separation of complex organic mixtures**, including reaction products, isomers, and multicomponent matrices.
5) **Identification** is achieved by retention-time comparison, detector selectivity, and GC–MS.
6) **Quantification** is based on peak area or height using external or internal standard methods.
7) GC integrates separation, identification, and quantification into a **single analytical workflow**.

12.8. TECHNICAL TERMS:

1) **Gas Chromatography (GC)** – Chromatographic technique using a gas as the mobile phase.
2) **Retention Time (t)** – Time from sample injection to peak maximum.
3) **Dead Time (t_m)** – Time taken by an unretained component to reach the detector.
4) **Adjusted Retention Time (t')** – Net retention time excluding dead time.
5) **Stationary Phase** – Phase responsible for retention and separation.
6) **Carrier Gas** – Inert gas transporting analytes through the column.
7) **Temperature Programming** – Gradual increase of oven temperature during GC run.
8) **FID** – Detector measuring ions formed during combustion of organic compounds.
9) **ECD** – Detector sensitive to electronegative compounds.
10) **NPD** – Detector selective for nitrogen and phosphorus compounds.
11) **GC–MS** – Hyphenated technique combining GC separation with mass analysis.
12) **Peak Area** – Integrated detector response proportional to analyte concentration.
13) **External Standard Method** – Quantification using calibration standards.
14) **Internal Standard Method** – Quantification using a reference compound added to samples.

12.9 SELF-ASSESSMENT QUESTIONS:

A. Short Answer Questions:

1) Define retention time in gas chromatography.
2) What is dead time? Why is it important?
3) Write the relationship between t_r, t_m, and t'_r.
4) List factors affecting retention time.
5) Why is retention time considered system-dependent?
6) State the role of retention time in qualitative GC analysis.
7) What is the significance of peak area in GC?

B. Descriptive / Essay Questions:

1) Explain retention time and its importance in gas chromatography.
2) Discuss factors affecting retention time with suitable examples.
3) Describe dead time and adjusted retention time with equations.
4) Explain the applications of GC in the separation of organic compounds.
5) Discuss methods of identification of organic compounds using GC.
6) Explain quantitative analysis in GC using external and internal standard methods.
7) Describe the integrated role of GC in separation, identification, and quantification.

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LESSON-13

ION EXCHANGE CHROMATOGRAPHY: PRINCIPLES AND PREPARATION OF ION-EXCHANGE RESINS

13.0. OBJECTIVES:

After studying this lesson, the student will be able to:

1) Understand the **principle and scope of ion exchange chromatography** as a separation technique.
2) Explain the **nature of the stationary phase and mechanism of ion exchange**.
3) Identify and discuss the **factors governing separation** in ion exchange chromatography.
4) Describe the **preparation of cross-linked polystyrene–divinylbenzene resins**.
5) Understand the **introduction of functional ionic groups** and the role of cross-linking in resin performance.
6) Correlate resin structure and properties with **selectivity, capacity, and efficiency** of separation.

STRUCTURE:

13.1 Introduction to Ion Exchange Chromatography

13.2 Basic Principles of Ion Exchange Chromatography

13.2.1. Definition and Scope

13.2.2. Nature of the Stationary Phase

13.2.3. Mechanism of Ion Exchange

13.2.4. Factors Governing Separation

13.3 Preparation of Cross-Linked Polystyrene Resins

13.3.1. Method of Preparation

13.3.2. Introduction of Functional Ionic Groups

13.3.3. Effect of Degree of Cross-Linking

13.4 Summary

13.5 Technical Terms

13.6 Self-Assessment Questions

13.7 Reference Text Books

13.1. INTRODUCTION TO ION EXCHANGE CHROMATOGRAPHY:

Ion Exchange Chromatography is an important and widely used separation and purification technique based on the reversible exchange of ions between a liquid phase and an insoluble solid matrix. It is particularly effective for the separation of ionic and ionizable compounds, including inorganic ions, organic acids, bases, amino acids, and biomolecules. Owing to its high selectivity, simplicity, and applicability over a wide range of conditions, ion exchange chromatography occupies a significant place in analytical chemistry, industrial purification processes, and biochemical analysis.

The technique employs a cross-linked polymeric resin containing fixed charged functional groups as the stationary phase. These fixed charges are balanced by mobile counter-ions, which can be exchanged with ions present in the sample solution. Separation is achieved because different ions exhibit varying affinities toward the resin depending on factors such as charge, size, hydration, and solution pH. By controlling experimental parameters such as pH and ionic strength, selective retention and elution of target species can be achieved.

Ion exchange chromatography is extensively used in the purification of carboxylic acids and amines, where differences in ionic character are exploited for efficient separation. The versatility of the technique, combined with the availability of a wide range of cation and anion exchange resins, makes it an indispensable tool in both laboratory-scale and large-scale purification processes.

13.2. BASIC PRINCIPLES OF ION EXCHANGE CHROMATOGRAPHY:

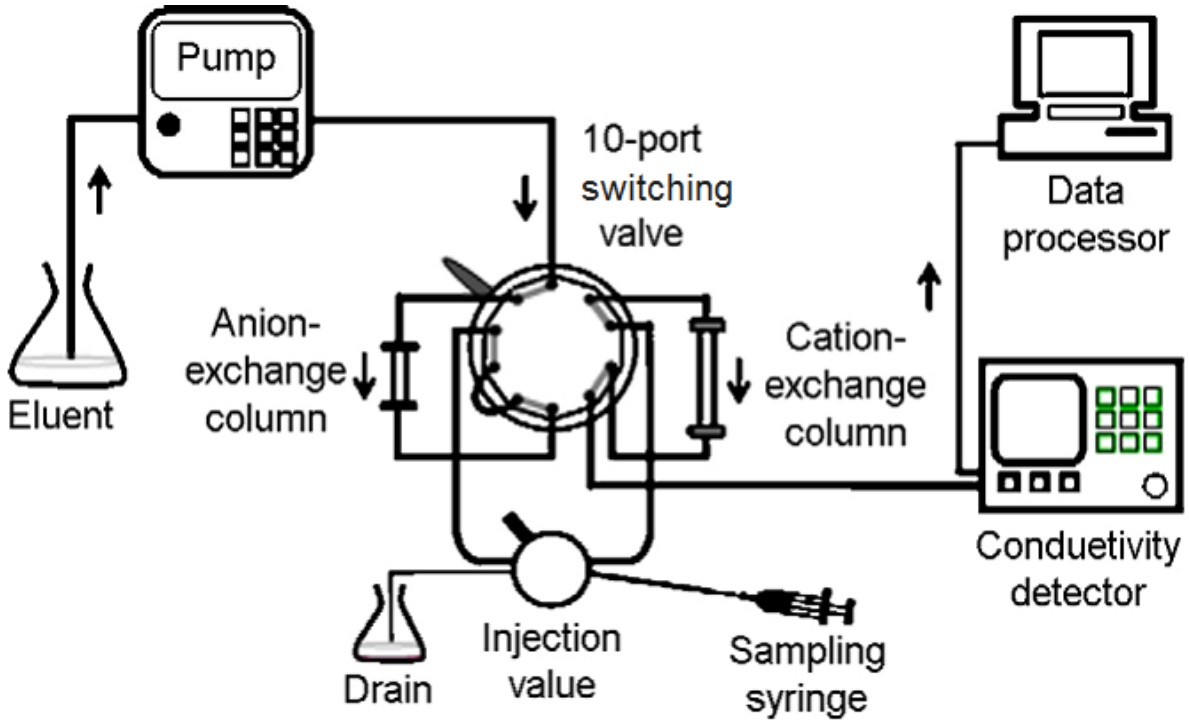


Figure 13.1: Schematic Diagram of an Ion Chromatography (Ion Exchange Chromatography) System

13.2.1. Definition and Scope:

Ion Exchange Chromatography (IEC) is an important liquid chromatographic separation technique employed for the separation, purification, and analysis of ionic and ionizable compounds. The technique is based on the reversible exchange of ions between ions present in the liquid phase (mobile phase) and oppositely charged ions bound to an insoluble solid matrix, known as the ion-exchange resin.

13.2.2. Nature of the Stationary Phase:

The stationary phase in ion exchange chromatography consists of a cross-linked polymeric resin containing fixed ionic functional groups. These functional groups may carry either negative charges (in cation exchange resins) or positive charges (in anion exchange resins). The fixed charges are electrically balanced by mobile counter-ions (such as H^+, Na^+, Cl^-, or OH^-), which are loosely held and can be replaced by ions of the same charge from the sample solution.

13.2.3. Mechanism of Ion Exchange:

When a solution containing ionic or ionizable species is passed through the ion-exchange column, an equilibrium is established between the ions in the solution and the counter-ions on the resin. Ions having greater affinity for the resin displace the counter-ions and become temporarily retained on the stationary phase. Ions with lower affinity interact weakly and pass through the column more rapidly.

The exchange process is reversible, allowing retained ions to be subsequently removed (eluted) by changing the pH, ionic strength, or composition of the mobile phase. Thus, separation is achieved due to differences in the strength of interaction between individual ions and the resin.

13.2.4. Factors Governing Separation:

The efficiency and selectivity of ion exchange chromatography depend on several factors, including:

i) **Nature and charge of the ions:** Ions with higher charge generally exhibit stronger interaction with the resin.

ii) **Ionic radius and degree of hydration:** Smaller and less hydrated ions are exchanged more readily.

iii) **pH of the mobile phase:** Controls the ionization state of analytes and functional groups on the resin.

iv) **Ionic strength of the mobile phase:** Higher ionic strength reduces retention by competing with sample ions.

v) **Type and capacity of the ion-exchange resin:** Determines selectivity, exchange capacity, and separation efficiency.

13.3. PREPARATION OF CROSS-LINKED POLYSTYRENE RESINS:

Ion-exchange resins used in ion exchange chromatography are most commonly based on cross-linked polystyrene matrices. These resins are preferred because of their chemical stability, mechanical strength, and ease of functionalization. The basic polymer framework is prepared by the copolymerization of styrene with divinylbenzene (DVB).

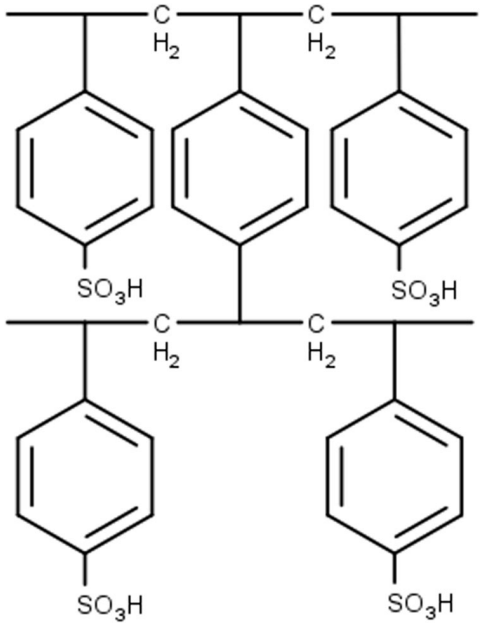


Figure 13.2: Structure of a Crosslinked Polystyrene Divinylbenzene Ion Exchange Resin

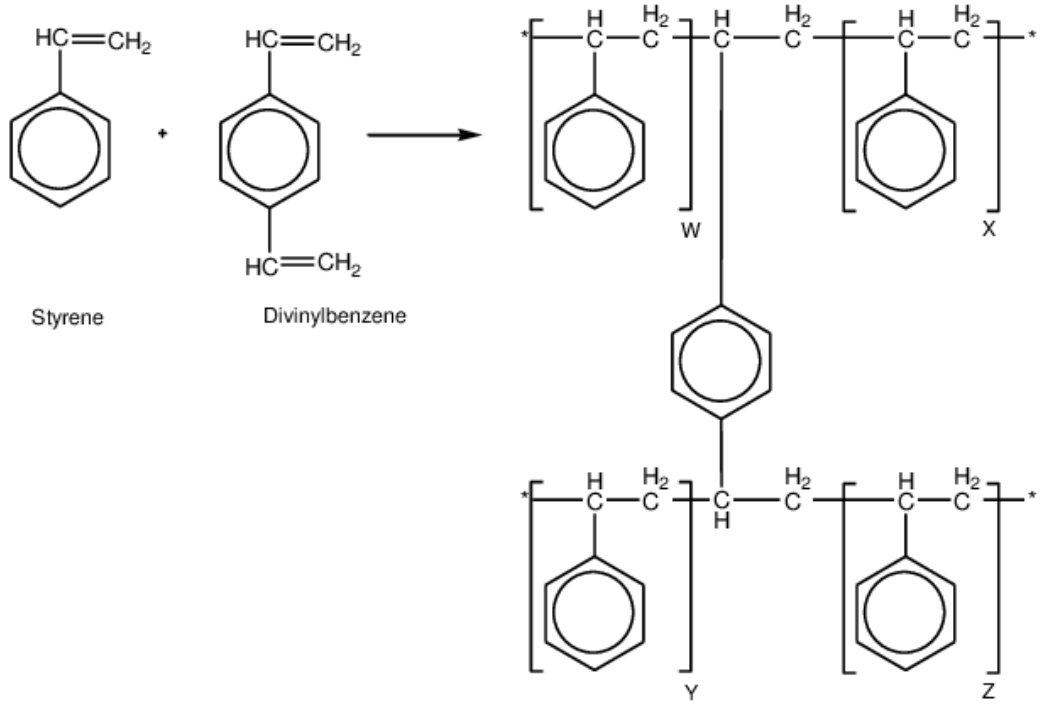


Figure 13.3: Copolymerization of Styrene and Divinyl Benzene Makes Polystyrene Resins

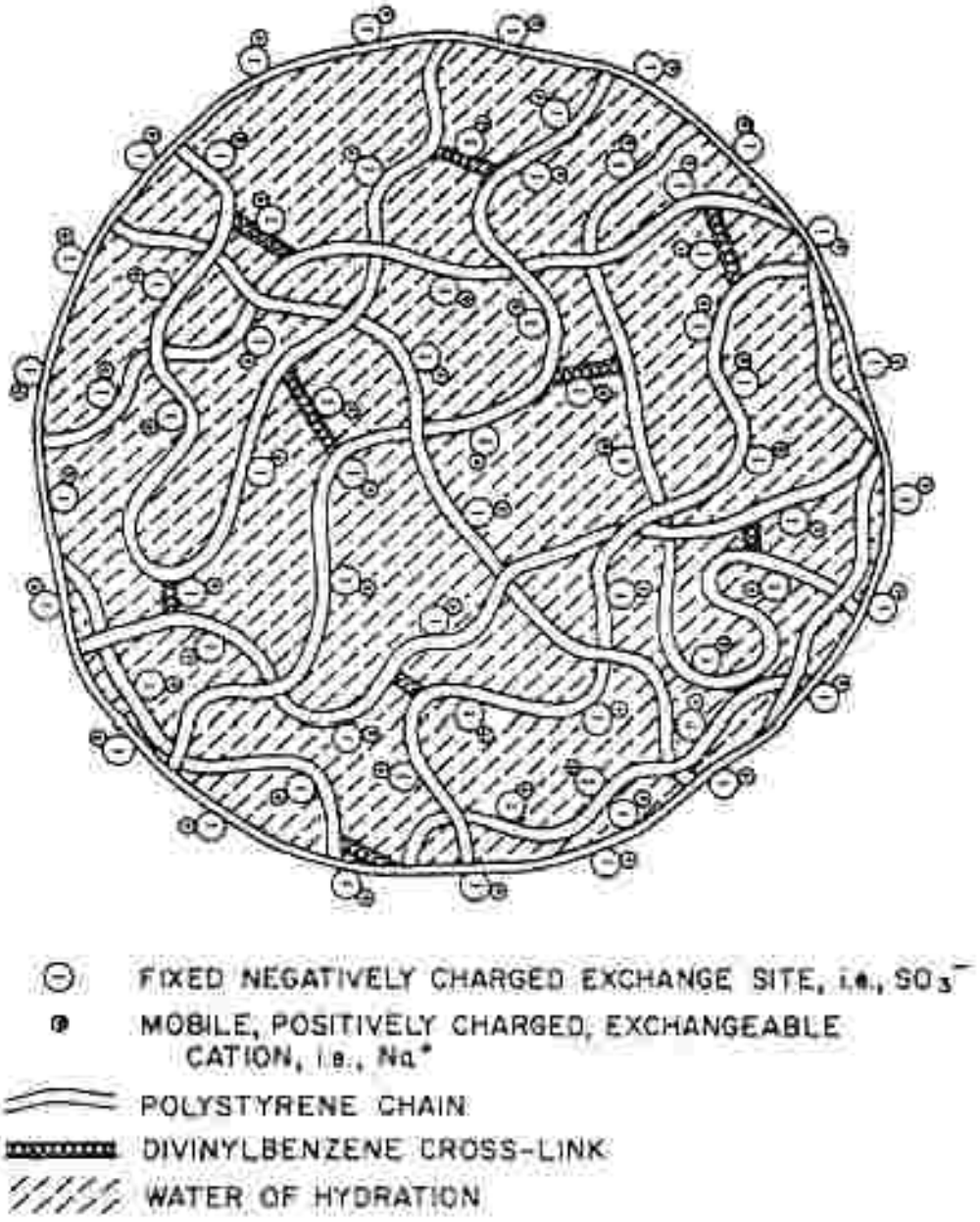


Figure 13.4.: Expanded view of a Polystyrene Bead

13.3.1. Method of Preparation:

Styrene undergoes free-radical polymerization in the presence of divinylbenzene, which acts as a cross-linking agent. During polymerization, divinylbenzene forms bridges between polystyrene chains, producing a three-dimensional cross-linked network. The polymerization is usually carried out in suspension, resulting in the formation of spherical polymer beads of uniform size.

The degree of cross-linking, typically in the range of 2–10% DVB, plays a crucial role in determining the physical properties of the resin. Low cross-linking produces softer, highly swollen resins with higher ion mobility, whereas higher cross-linking yields more rigid, mechanically strong resins with lower swelling capacity.

13.3.2. Introduction of Functional Ionic Groups:

The cross-linked polystyrene matrix obtained after polymerization is chemically inert and insoluble. To convert it into an ion-exchange resin, suitable ionic functional groups are introduced into the polymer backbone through specific chemical reactions.

- **Sulfonation** of the polystyrene matrix introduces $–SO_3H$ (sulfonic acid) groups, producing strong acid cation-exchange resins capable of exchanging hydrogen ions with metal or organic cations.
- **Chloromethylation** followed by amination introduces amine or quaternary ammonium groups, resulting in anion-exchange resins. Depending on the type of amine introduced, either weak-base or strong-base anion exchangers can be obtained.

These functional groups are covalently bound to the polymer matrix, ensuring stability during repeated ion-exchange cycles.

13.3.3. Effect of Degree of Cross-Linking:

The extent of cross-linking significantly influences the performance of ion-exchange resins:

- **Ion-exchange capacity:** Lower cross-linking generally increases accessibility of functional groups, enhancing capacity.
- **Rate of ion diffusion:** Less cross-linked resins allow faster diffusion of ions into and out of the resin beads.
- **Selectivity and stability:** Higher cross-linking improves mechanical strength, thermal stability, and resistance to chemical degradation.

Therefore, an optimum degree of cross-linking is selected based on the intended application and operating conditions.

13.4 SUMMARY:

1) Ion exchange chromatography is based on the **reversible exchange of ions** between a liquid phase and an insoluble solid resin.
2) The stationary phase consists of **cross-linked polymeric resins** bearing fixed charged functional groups balanced by mobile counter-ions.
3) Separation occurs because different ions show **different affinities toward the resin**, depending on charge, size, hydration, and pH.
4) The most widely used ion-exchange resins are based on **polystyrene cross-linked with divinylbenzene (DVB)**.
5) Functional groups such as **$–SO_3H$** (cation exchangers) and **amine or quaternary ammonium groups** (anion exchangers) are introduced chemically.
6) The **degree of cross-linking** strongly affects ion-exchange capacity, diffusion rate, selectivity, and mechanical stability.
7) Ion exchange chromatography is extensively applied in the **purification of inorganic ions, carboxylic acids, amines, and biomolecules**.

13.5. TECHNICAL TERMS:

1) **Ion Exchange Chromatography (IEC)** – A liquid chromatographic technique based on reversible exchange of ions.
2) **Ion-Exchange Resin** – An insoluble polymer matrix containing fixed ionic groups.
3) **Stationary Phase** – Cross-linked polymeric resin bearing charged functional groups.
4) **Mobile Phase** – Liquid containing ions that undergo exchange with the resin.
5) **Counter-Ion** – Ion associated with the fixed charge on the resin and exchangeable with sample ions.
6) **Cation Exchange Resin** – Resin containing negatively charged groups that exchange cations.
7) **Anion Exchange Resin** – Resin containing positively charged groups that exchange anions.
8) **Polystyrene–DVB Resin** – Common polymer matrix used for ion-exchange resins.
9) **Divinylbenzene (DVB)** – Cross-linking agent used in resin preparation.
10) **Cross-Linking** – Formation of a three-dimensional polymer network by covalent bonding.
11) **Sulfonation** – Chemical introduction of $–SO_3H$ groups into polystyrene.
12) **Chloromethylation** – Reaction introducing $–CH_2Cl$ groups for subsequent amination.
13) **Ion-Exchange Capacity** – Number of ions a resin can exchange per unit mass.
14) **Ionic Strength** – Measure of total ion concentration in the mobile phase.
15) **Hydration** – Association of ions with water molecules affecting exchange behaviour.

13.6. SELF-ASSESSMENT QUESTIONS:

A. Short Answer Questions

1) Define ion exchange chromatography.
2) What is meant by an ion-exchange resin?
3) Distinguish between cation and anion exchange resins.
4) What role does divinylbenzene play in resin preparation?
5) State any two factors affecting ion exchange separation.
6) Why are polystyrene-based resins widely used?
7) What is the significance of pH in ion exchange chromatography?

B. Descriptive / Essay Questions:

1) Explain the **basic principles and mechanism** of ion exchange chromatography.
2) Describe the **nature of the stationary phase** used in ion exchange chromatography.
3) Discuss the **factors governing separation** in ion exchange chromatography.
4) Explain the **preparation of cross-linked polystyrene–divinylbenzene resins**.
5) Describe the **introduction of functional ionic groups** into polystyrene resins.
6) Discuss the **effect of degree of cross-linking** on resin properties and performance.
7) Write a detailed note on the **applications of ion exchange chromatography**.

13.7. REFERENCE TEXT BOOKS:

1) **Skoog, D.A., Holler, F.J., & Crouch, S.R.** *Principles of Instrumental Analysis*, 6th Edition, Cengage Learning.
2) **Christian, G. D.** *Analytical Chemistry*, 6th Edition, John Wiley & Sons.
3) **Willard, H.H., Merritt, L.L., Dean, J.A., & Settle, F.A.** *Instrumental Methods of Analysis*, 7th Edition, CBS Publishers.
4) **Vogel, A.I.** *Textbook of Quantitative Chemical Analysis*, 6th Edition, Pearson Education.
5) **Skoog, D.A., West, D.M., Holler, F.J., & Crouch, S.R.** *Fundamentals of Analytical Chemistry*, 9th Edition, Cengage Learning.

Dr. P. Bharath

LESSON-14

TYPES OF ION EXCHANGE RESINS AND THEIR APPLICATIONS

14.0. OBJECTIVES:

After studying this lesson, the student will be able to:

1) Classify **ion exchange resins** based on the nature and charge of functional groups.
2) Distinguish between **cation and anion exchange resins**.
3) Explain the **structure, functional groups, and ion-exchange mechanisms** of strong and weak resins.
4) Compare **strong acid vs weak acid cation exchangers** and **strong base vs weak base anion exchangers**.
5) Describe the **applications of ion exchange resins** in purification of carboxylic acids and amines.
6) Understand the **advantages and limitations** of ion exchange chromatography.

STRUCTURE:

14.1 Types of Ion Exchange Resins

14.2 Cation Exchange Resins

14.2.1 Strong Acid Cation Exchange Resins

14.2.2 Weak Acid Cation Exchange Resins

14.3 Anion Exchange Resins

14.3.1 Strong Base Anion Exchange Resins

14.3.2 Weak Base Anion Exchange Resins

14.4 Applications

14.4.1 Applications in the Purification of Carboxylic Acids

14.4.2 Applications in the Purification of Amines

14.5 Advantages and Limitations of Ion Exchange Chromatography

14.6 Summary

14.7 Technical Terms

14.8 Self-Assessment Questions

14.9 Reference Text Books

14.1. TYPES OF ION EXCHANGE RESINS:

Ion exchange resins are broadly classified based on the nature and charge of the fixed ionic functional groups chemically bonded to the polymer matrix. These fixed functional

groups impart a permanent charge to the resin and determine whether the resin is capable of exchanging positively charged ions (cations) or negatively charged ions (anions) from the solution. In addition, the chemical nature and strength of these functional groups govern the exchange capacity, selectivity, and effective operating pH range of the resin.

Accordingly, ion exchange resins are categorized into cation exchange resins and anion exchange resins, each of which may further be classified as strong or weak depending on the degree of ionization of the functional groups. Proper classification and selection of ion exchange resins are essential for achieving efficient and selective separations in ion exchange chromatography and purification processes.

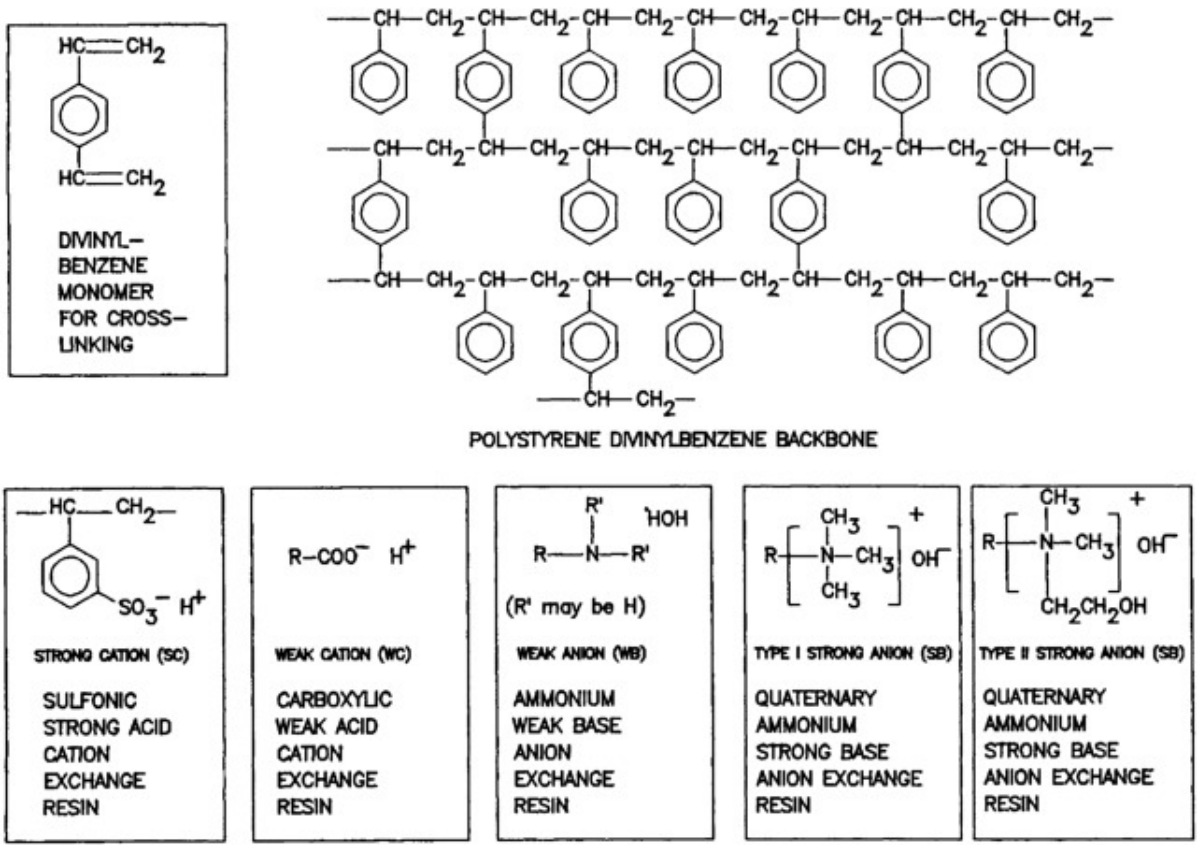


Figure 14.1: Ion Exchange Resins

14.2. CATION EXCHANGE RESINS:

Cation exchange resins contain **negatively charged functional groups** attached to the polymer backbone and are capable of exchanging **positively charged ions (cations)** present in the solution. These resins typically exchange hydrogen ions (H^+) or other counter-cations with metal ions or organic cations from the sample.

14.2.1. Strong Acid Cation Exchange Resins:

Strong acid cation exchange resins are ion exchange materials that contain **strongly acidic functional groups** covalently bonded to a polymeric matrix. These functional groups remain **fully ionized over a wide range of pH values**, enabling the resin to exhibit **uniform and consistent ion-exchange behavior under both acidic and alkaline conditions**.

The permanent ionization of the acidic groups ensures that the resin can effectively exchange cations irrespective of variations in solution pH, making these resins highly versatile and widely applicable.

Functional Group and Exchange Mechanism:

- **Functional group** **:** –SO_3H (sulfonic acid)
- **Operating pH range** **:** Effective over a wide pH range
- **Exchange mechanism** **:** Hydrogen ions (H^+) bound to the sulfonic acid groups are reversibly exchanged with **metal ions** such as Na^+, Ca^{2+}, and Mg^{2+}, or with **organic cations** present in the solution.

The sulfonic acid group is strongly acidic and remains dissociated, resulting in a fixed -**SO_3^- group** on the resin matrix, which facilitates rapid and efficient cation exchange.

Characteristics:

Strong acid cation exchange resins possess several advantageous properties:

- **High ion-exchange capacity**, enabling efficient removal of cations
- **Excellent chemical and thermal stability**, allowing operation under harsh conditions
- **Rapid and efficient ion-exchange kinetics**, leading to fast separations
- **Good mechanical strength**, making them suitable for column operation
- **Capability for repeated regeneration and reuse**, enhancing economic efficiency

Example:

A typical example of a strong acid cation exchange resin is **sulfonated polystyrene-divinylbenzene (DVB) resin**, in which sulfonic acid groups are introduced into a cross-linked polystyrene matrix.

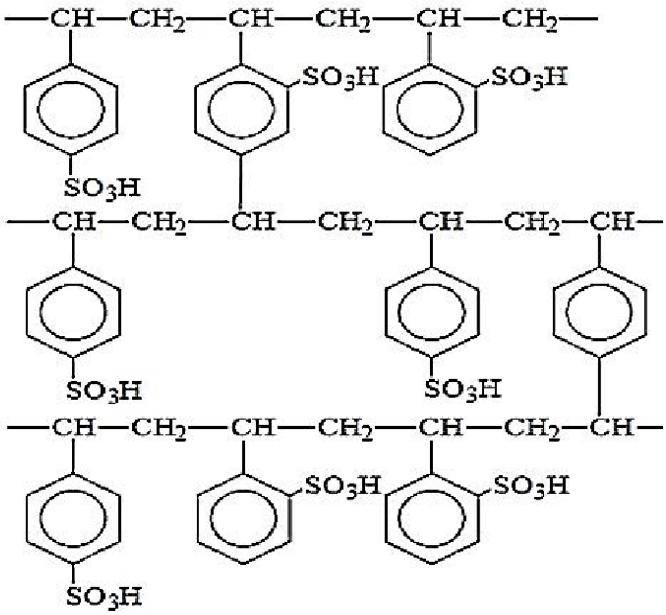


Figure 14.2: Chemical Structure of Sulfonated Polystyrene Resin

Applications:

Due to their robustness, high capacity, and broad pH applicability, strong acid cation exchange resins are extensively used in: *Water softening and demineralization, Purification and separation of amines, Removal of metal ions from industrial effluents and Separation of inorganic cations in analytical and industrial processes.*

14.2.2 Weak Acid Cation Exchange Resins:

Weak acid cation exchange resins contain **weakly acidic functional groups**, which are only partially ionized and become effective mainly at **higher (alkaline) pH values**. Consequently, their ion-exchange behavior is strongly **pH-dependent**.

- **Functional group** **:** –COOH (carboxylic acid)
- **pH range** **:** Effective mainly in alkaline conditions
- **Exchange capacity** **:** Lower than that of strong acid resins

Characteristics:

- Ionization depends on solution pH
- Greater selectivity for certain cations
- Less effective in strongly acidic media
- Lower overall exchange capacity

Example:

A typical weak acid cation exchange resin is a **carboxylated polystyrene–divinylbenzene resin**, in which carboxylic acid groups are introduced into a cross-linked polymer matrix.

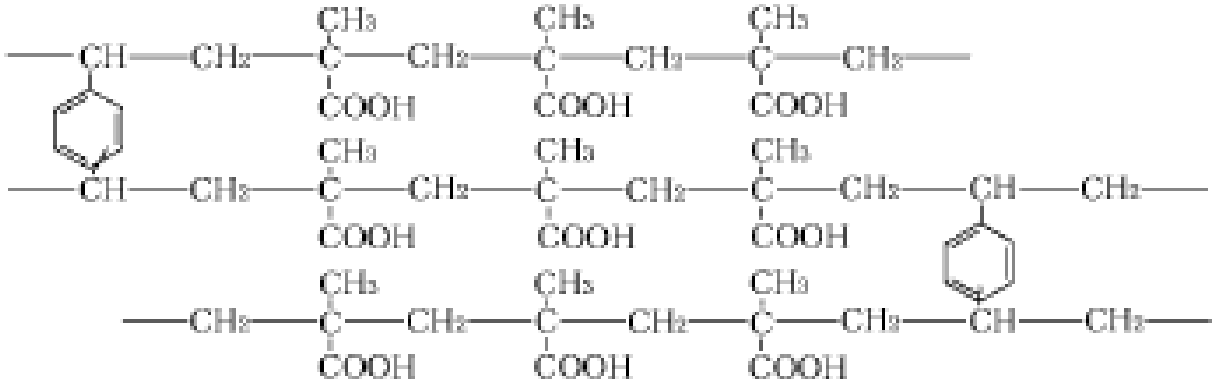


Figure 14.3: Weakly Acidic Cation Exchange Resin

Applications:

Weak acid cation exchange resins are particularly useful in applications requiring **selective separation of cations** under carefully controlled pH conditions. They are commonly employed in the **purification of specific metal ions**, **separation of organic**

cations, and **selective ion removal processes** where high selectivity is preferred over high capacity.

14.3. ANION EXCHANGE RESINS:

Anion exchange resins are ion exchange materials that contain **positively charged functional groups** attached to a polymeric matrix. These fixed cationic groups enable the resin to **exchange negatively charged ions (anions)** such as chloride, sulfate, nitrate, hydroxide, and various organic anions present in the solution. Depending on the strength of the basic functional groups, anion exchange resins are classified into **strong base** and **weak base anion exchangers**.

14.3.1 Strong Base Anion Exchange Resins:

Functional Group and Chemical Nature:

Strong base anion exchange resins possess **permanently charged quaternary ammonium groups (–NR_3^+)** covalently bonded to the polymer backbone. These groups remain ionized over a **wide range of pH values**, making the resin effective under both acidic and alkaline conditions.

Ion-Exchange Mechanism:

In their active form, strong base anion exchange resins usually contain **hydroxide ions (OH^-)** as counter-ions. During the ion-exchange process, these hydroxide ions are **reversibly exchanged with anions** such as Cl^-, SO_4^{2-}, NO_3^-, or organic anions present in the sample solution.

Characteristics:

Strong base anion exchange resins exhibit:

- **High ion-exchange capacity**
- Effectiveness over a **wide pH range**
- Ability to exchange both **strong and weak acid anions**
- **Excellent chemical stability** and good regeneration capability

Example: Quaternary ammonium polystyrene–divinylbenzene resin

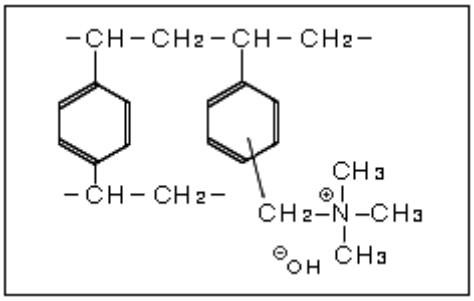


Figure 14.4: Polystyrene–DVB resin with –$N(CH_3)_3^+$ groups

Applications:

Because of their high capacity and broad applicability, strong base anion exchange resins are widely used in: *Water deionization and purification, Purification of carboxylic acids, Removal of inorganic anions from industrial and environmental samples*

14.3.2 Weak Base Anion Exchange Resins:

Functional Group and Chemical Nature

Weak base anion exchange resins contain **weakly basic amine groups**, such as **–NH_2, –NHR, and –NR_2**, attached to the polymer matrix. These functional groups are **protonated only under acidic or neutral conditions**, which makes their ion-exchange behavior **pH-dependent**.

Ion-Exchange Mechanism:

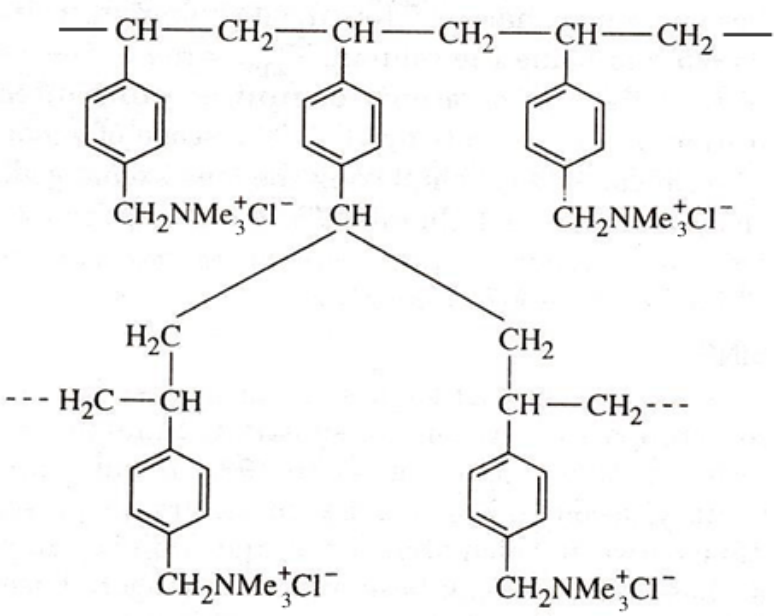
Under acidic or neutral conditions, the amine groups become protonated to form positively charged sites that can **exchange anions** from the solution. In alkaline media, these groups lose their positive charge, and the resin becomes largely inactive.

Characteristics:

Weak base anion exchange resins are characterized by:

- **Lower exchange capacity** compared to strong base resins
- **pH-dependent ion-exchange behavior**
- Ineffectiveness in strongly alkaline solutions
- **Selective removal of weak acid anions**

Example: Amine-functionalized polystyrene–DVB resin



14.4. APPLICATIONS:

Weak base anion exchangers are commonly employed in: *Purification of organic acids, Selective anion separations, Processes where controlled and selective exchange is preferred.*

14.4.1. Applications in the Purification of Carboxylic Acids:

Principle:

Carboxylic acids, when present in **alkaline solutions**, dissociate to form **negatively charged carboxylate ions ($RCOO^-$)**. These anions can be selectively separated using **anion exchange resins**, which contain positively charged functional groups capable of binding negatively charged species. The separation is based on the **reversible ion-exchange interaction** between carboxylate ions and the resin.

Ion-Exchange Mechanism:

In anion exchange chromatography, the resin is commonly used in the **hydroxide (OH^-) form**. When a solution containing carboxylate ions passes through the column, the **$RCOO^-$ ions replace the OH^- ions** associated with the resin and become bound to the fixed positive sites. Neutral and non-ionic impurities do not interact with the resin and pass through the column unretained.

Process of Purification:

i) **Loading of Sample**

The alkaline solution containing carboxylate ions is passed through a column packed with an **anion exchange resin in the OH^- form**.

ii) **Retention of Carboxylate Ions**

The negatively charged **carboxylate ions ($RCOO^-$)** displace OH^- ions and bind to the resin through electrostatic attraction.

iii) **Removal of Impurities**

Neutral molecules and weakly interacting impurities are **washed out of the column** using an appropriate solvent or buffer.

iv) **Elution and Recovery**

The bound carboxylate ions are released by **elution with a suitable acid**. The acid reprotonates the carboxylate ions, converting them back into the **free carboxylic acid (RCOOH)**, which is collected in purified form.

Advantages of the Method:

- High **selectivity for carboxylate ions**
- Mild operating conditions
- Efficient removal of neutral and ionic impurities
- Suitable for both laboratory and industrial-scale purification

Applications:

This ion exchange method is widely used in the purification of:

- Organic carboxylic acids
- Fatty acids
- **Fermentation products** such as lactic acid, citric acid, and acetic acid
- Carboxylic acid intermediates in pharmaceutical and biochemical industries

14.4.2 Applications in the Purification of Amines:

Principle:

Amines are basic compounds that, in **acidic solutions**, readily accept protons to form **positively charged ammonium ions (RNH_3^+)**. These protonated amines can be selectively separated using **cation exchange resins**, which contain negatively charged functional groups capable of binding cations through electrostatic attraction.

Ion-Exchange Mechanism:

Cation exchange resins are commonly used in the **hydrogen (H^+) form**. When a solution containing protonated amines is passed through the column, the **RNH_3^+ ions replace the H^+ ions** associated with the resin and become bound to the fixed anionic sites. Neutral and non-basic components do not interact significantly with the resin and therefore pass through the column.

Process of Purification:

i) **Sample Loading**

The acidic solution containing protonated amines is passed through a column packed with a **cation exchange resin in the H^+ form**.

ii) **Retention of Amines**

The positively charged **RNH_3^+ ions** displace H^+ ions and bind to the resin through ionic interactions.

iii) **Removal of Impurities**

Neutral, non-basic, and weakly interacting impurities are **washed away** using an appropriate solvent or buffer.

iv) **Elution and Recovery**

The bound amines are recovered by **elution with a basic solution**. The base neutralizes the ammonium ions, converting them back to free amines (RNH_2), which are released from the resin and collected in purified form.

Applications:

Ion exchange chromatography is extensively used for the purification of:

- Aliphatic and aromatic amines
- Alkaloids
- Amino acids and peptides
- Pharmaceutical intermediates and drug substances

14.5. ADVANTAGES AND LIMITATIONS OF ION EXCHANGE CHROMATOGRAPHY:

Advantages:

Ion exchange chromatography offers several important advantages:

- **High selectivity for ionic and ionizable species**, leading to efficient separations
- **Reusable resins**, which can be regenerated and employed repeatedly
- **Suitability for large-scale purification**, including industrial applications
- **Mild operating conditions**, preserving sensitive compounds

Limitations

Despite its advantages, the technique has certain limitations:

- **Not suitable for non-ionic compounds**, as separation depends on charge
- **Resin fouling or degradation** may occur due to strongly adsorbed impurities
- **Performance is highly dependent on pH and ionic strength**, requiring careful control of operating conditions

14.6 SUMMARY:

1) Ion exchange resins are classified into **cation exchange resins** and **anion exchange resins** based on the charge of fixed functional groups.
2) Cation exchangers contain **negatively charged groups** and exchange cations, while anion exchangers contain **positively charged groups** and exchange anions.
3) Both cation and anion exchangers are further classified as **strong or weak** depending on the degree of ionization of their functional groups.
4) **Strong acid cation exchangers** ($–SO_3H$) operate over a wide pH range and have high capacity.
5) **Weak acid cation exchangers** (–COOH) show pH-dependent behavior and higher selectivity.
6) **Strong base anion exchangers** ($–NR_3^+$) exchange both strong and weak acid anions over a wide pH range.
7) **Weak base anion exchangers** ($–NH_2$, –NHR, $–NR_2$) provide controlled and selective anion exchange.
8) Ion exchange chromatography is widely applied in the **purification of organic acids, amines, metal ions, and pharmaceutical intermediates**.
9) The technique offers high selectivity and mild conditions but is limited to ionic species and is sensitive to pH and ionic strength.

14.7. TECHNICAL TERMS:

1) **Ion Exchange Resin** – Insoluble polymer containing fixed ionic groups.
2) **Cation Exchange Resin** – Resin that exchanges positively charged ions.

3) **Anion Exchange Resin** – Resin that exchanges negatively charged ions.
4) **Strong Acid Cation Exchanger** – Resin with fully ionized $–SO_3H$ groups.
5) **Weak Acid Cation Exchanger** – Resin containing –COOH groups with pH-dependent ionization.
6) **Strong Base Anion Exchanger** – Resin with quaternary ammonium ($–NR_3^+$) groups.
7) **Weak Base Anion Exchanger** – Resin with amine groups ionized only under acidic conditions.
8) **Polystyrene–DVB Resin** – Common polymer matrix used in ion exchange resins.
9) **Divinylbenzene (DVB)** – Cross-linking agent providing mechanical strength.
10) **Ion-Exchange Capacity** – Quantity of ions exchanged per unit mass of resin.
11) **Counter-Ion** – Exchangeable ion associated with the fixed charge on the resin.
12) **Regeneration** – Restoration of resin to its active ionic form.
13) **Carboxylate Ion ($RCOO^-$)** – Anionic form of carboxylic acids.
14) **Ammonium Ion (RNH_3^+)** – Protonated form of amines.

14.8. SELF-ASSESSMENT QUESTIONS:

A. Short Answer Questions:

1) Define ion exchange resin.
2) Classify ion exchange resins.
3) What is a strong acid cation exchange resin?
4) Give the functional group of weak acid cation exchangers.
5) What is the functional group of strong base anion exchangers?
6) Why are weak base anion exchangers pH-dependent?
7) State two applications of ion exchange chromatography.

B. Descriptive / Essay Questions:

1) Explain the classification of ion exchange resins with examples.
2) Describe strong acid cation exchange resins in detail.
3) Compare strong acid and weak acid cation exchange resins.
4) Explain strong base and weak base anion exchange resins.
5) Describe the purification of carboxylic acids using ion exchange chromatography.
6) Explain the purification of amines using cation exchange resins.
7) Discuss the advantages and limitations of ion exchange chromatography.

14.9. REFERENCE TEXT BOOKS:

1) **Skoog, D. A., Holler, F. J., & Crouch, S. R.** *Principles of Instrumental Analysis*, 6th Ed., Cengage Learning.

2) **Christian, G.D.** *Analytical Chemistry*, 6th Ed., John Wiley & Sons.

3) **Willard, H.H., Merritt, L.L., Dean, J.A., & Settle, F.A.** *Instrumental Methods of Analysis*, 7th Ed., CBS Publishers.

4) **Vogel, A.I.** *Textbook of Quantitative Chemical Analysis*, 6th Ed., Pearson Education.

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Dr. P. Bharath

LESSON-15

ATOMIC ABSORPTION SPECTROSCOPY

15.0. OBJECTIVES:

After studying this lesson, you should be able to:

- To know about the Atomic Absorption Spectroscopy.
- To study about Principle involved in AAS.
- To study about Instrumentation of AAS.
- To study about Advantages, Disadvantages and Applications of AAS.

STRUCTURE:

15.1 Introduction

15.2 Principle

15.3 Working Principle

15.4 Instrumentation of AAS

15.5 Advantages of AAS

15.6 Disadvantages of AAS

15.7 Limitations of AAS

15.8 Applications of AAS

15.9 Summary

15.10 Self-Assessment Questions

15.11 Reference Books

15.1. INTRODUCTION:

AAS is a technique used for determining the concentration of a particular metal element within a sample. AAS is used to analyse the concentration of over 62 different metals in a solution. AAS is a method of analysis based on absorption of radiation by atoms when a solution of metallic salt is aspirated (drawing) into a flame. Only a drop of sample needed. The metals need not be removed from other components. Sensitive in the ppm range.

Atomic absorption spectroscopy (AAS) is specifically designed for the analysis of the metals and metalloids substances. AAS is a quantitative analytical technique wherein the absorption of a specific wavelength of radiation by the neutral atoms in the ground state is measured. The more the number of the atoms in each sample, the higher is the intensity of absorption and vice-versa. This is also known as ***metal analysis spectroscopy*** as it is mainly used for the analysis of metals.

15.2. PRINCIPLE:

AAS method is like that of spectrophotometer. The only exception is the replacement of the sample cell by a flame.

Atoms (and ions) can absorb light at a specific, unique wavelength. When this specific wavelength of light is provided, the energy (light) is absorbed by the atom.

- Electrons in the atom move from the ground state to an excited state.
- The amount of light absorbed is measured and the concentration of the element in the sample can be calculated.
- The radiant energy absorbed by the electrons is directly related to the transition that occurs during this process.

Furthermore, since the electronic structure of every element is unique, the radiation absorbed represents a unique property of each individual element and it can be measured.

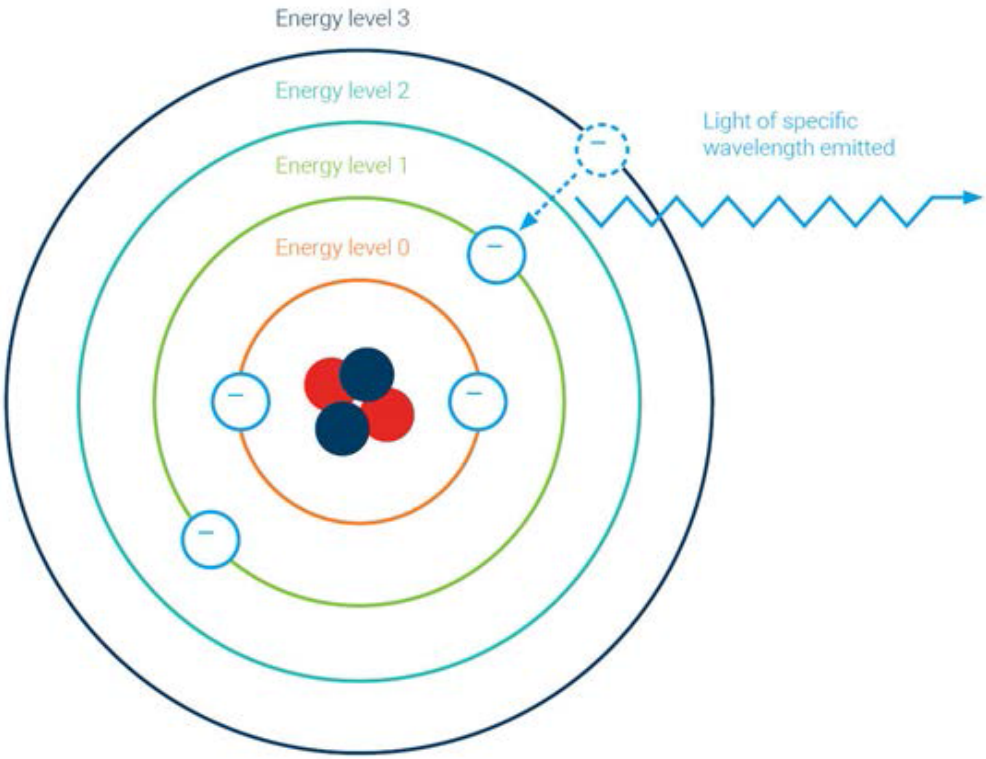


Figure 1: An Electron is Excited from the Ground State to Higher Energy Level by Absorbing Energy (Light)

15.3. WORKING PRINCIPLE:

In AAS, a monochromatic light for a particular element is produced by a hollow cathode lamp utilizing that element as the cathode. The monochromatic light produced by the lamp is beamed through a long flame into which is aspirated the solution to be analysed. The heat energy dissociates the molecules and converts the components to atoms. At flame temperature, some atoms in the solution are activated, but most of the atoms are remain in the ground state.

The ground state atoms of the same element as in the hollow cathode cup absorb their own resonance (reflected) lines. The amount of light absorbed varies directly with their concentration in the flame. The transmitted light that is not absorbed reaches the monochromator. The monochromator passes only the wavelengths close to the resonance lines of the element to be analysed. A monochromator is placed between the sample and the detector to reduce back ground interference. Then the transmitted light strikes a detector and the decrease in transmitted light is measured. From here, the detector measures the intensity of the beam of light and converts it to absorption data.

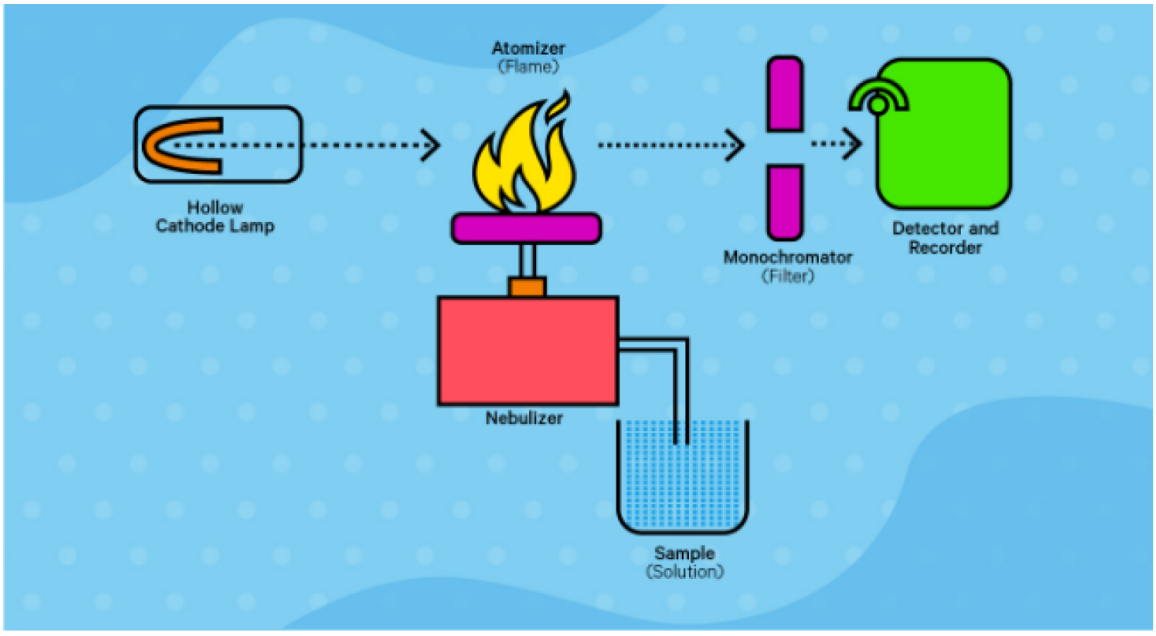


Figure 2: Schematic Representation of a Typical AAS.

15.4. INSTRUMENTATION OF AAS:

The Atomic absorption spectroscopy has simple instrumentation. But, unlike other spectroscopic methods, it has two additional requirements. These include a specially designed lamp to produce light of a desired wavelength and a burner to prepare the sample for absorption of light radiation. Additionally, the instrument also sprays the sample in the solution state over an atomizer (burner). This leads to evaporation of the solvent and leaves a fine dry residue. This residue has neutral atoms in the ground state. There

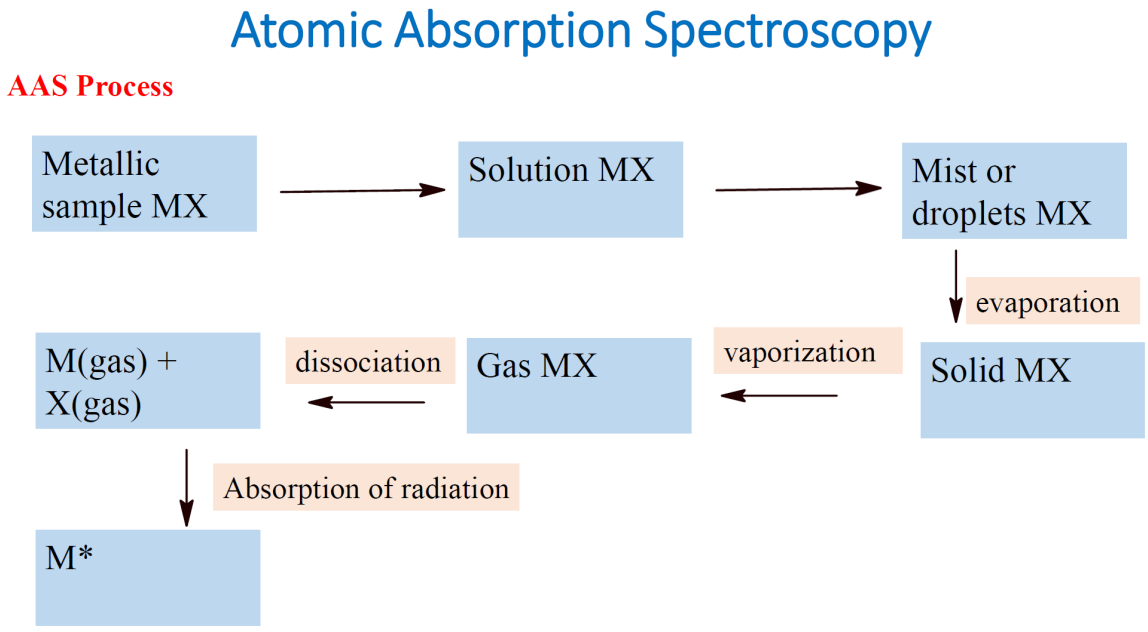


Figure 3: AAS Process

Components in AAS Instrument:

1) Radiation source
2) Chopper/ Sputtering
3) Atomizers
4) Nebuliser
5) Monochromator
6) Detector
7) Amplifier
8) Read out device

1. Radiation Source:

Halo Cathode Lamp:

The most widely used light source is the hollow cathode lamp. These lamps are designed to emit the atomic spectrum of a particular element, and specific lamps are selected for use depending on the element to be determined.

Metal	Zn	Fe	Cu	Ca	Na
λ_{Max}	214	248	325	423	589

The cathode of the lamp is a hollow-out cylinder of the metal whose spectrum is to be produced. Each analyzed element requires a different lamp. The anode and cathode are sealed

in a glass cylinder normally filled with either neon or argon at low temperature. At the end of the glass cylinder is window transparent to the emitted radiation. The cathode lamps are stored in a compartment inside the AA spectrometer. The specific lamp needed for a given metal analysis is rotated into position for a specific experiment.

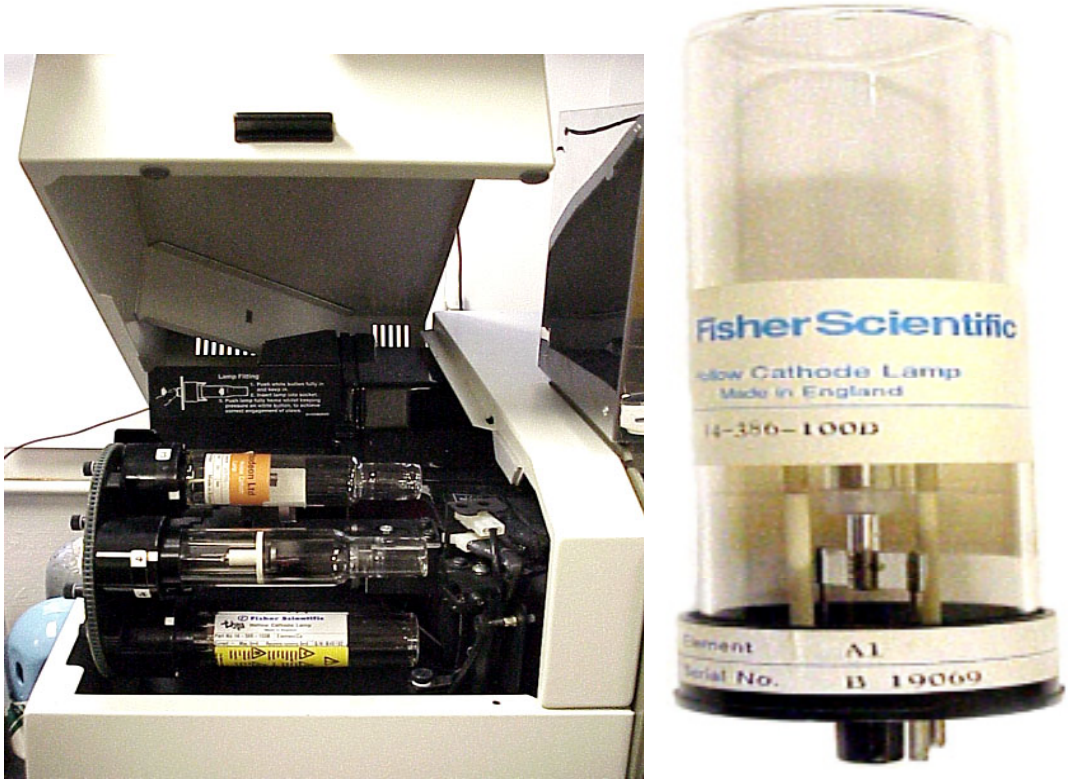
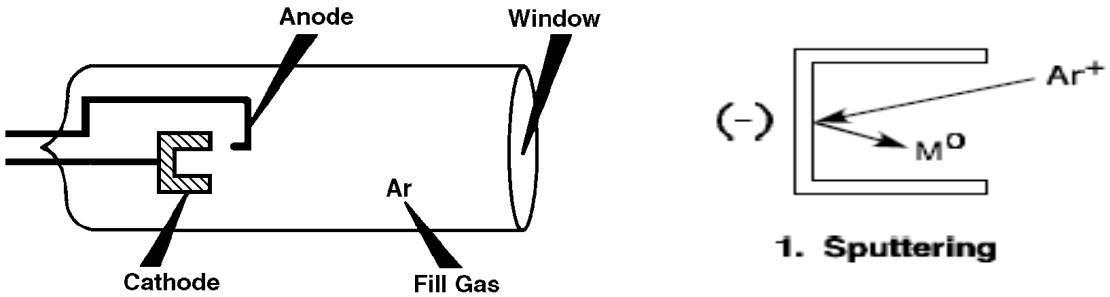


Figure 4: Halo Cathode Lamp and in Instrument Place

Chopper:

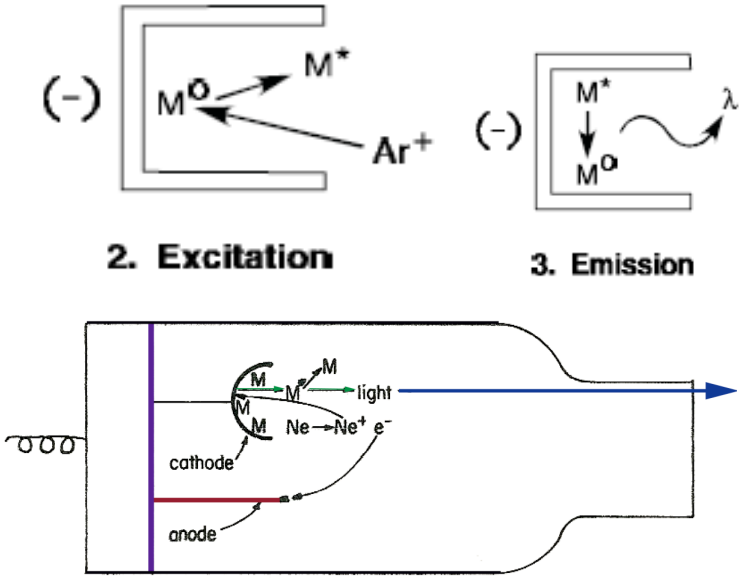
- When an electrical potential is applied between the anode and cathode, some of the fill gas atoms are ionized. The positively charged fill gas ions accelerate through the electrical filed (gather in a line) to collide with the negatively charged cathode and dislodge individual metal atoms in a process called sputtering.



Excitation and Emission:

- Then the sputtered metal atoms are elevated to an excited state.
- When the atoms return to the ground state, the characteristic line spectrum (light) of that atom is emitted.

- Then the emitted light is directed at the flame where unexcited atoms of the same element absorb the radiation and are themselves raised to the excited state.
- Then the absorbance is measured.



2. Beam Chopper:

- In AAS, the hollow cathode lamp and flame are light emitting source.
- The phototube responds to radiation from the hollow cathode lamp as well as flame.
- This will create interference in absorption measurement.
- This problem is corrected by a beam chopper.
- Beam chopper is a motor driven device that has open and solid (or mirrors in some cases) alternating regions.
- One half of their rotation, i.e., **open region**, permits the beam obtained from lamp to pass through.
- During the other half of their rotation, i.e., **mirror region**, the beam is reflected and not allowed to pass through.

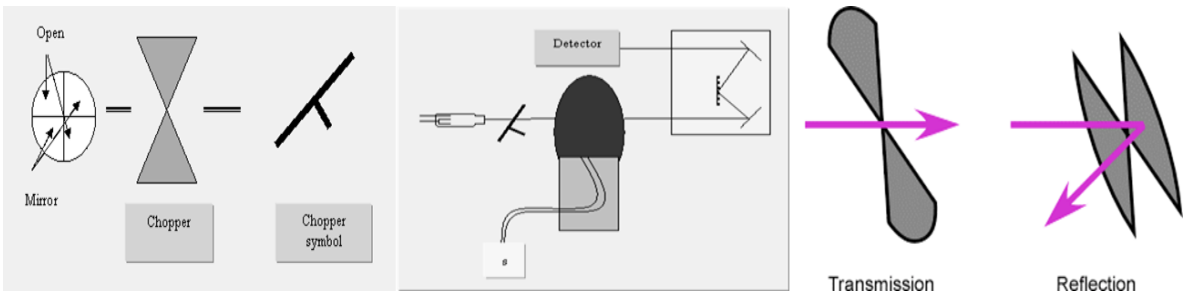


Figure 5: Represenattion of Light Beam on Chopper

3. The Flame / Burner System:

The third unique component in the system is the burner, through which the sample is introduced. The burner consists of three parts, namely,

- nebulizer,
- premix chamber
- burner head.

Job of the Flame:

- Destroy any analyte ions and breakdown complexes.
- Create atoms (the elemental form) of the element of interest. FeO, CuO, ZnO etc.

Premix Chamber:

- Sample solution, fuel gas and oxidising gas are mixed in mixing chamber
- This mixture is then passed through a series of baffles where thorough mixing and formation of uniform droplets of the sample take place.
- The **nebulizer chamber** / **premix chamber** thoroughly mixes acetylene (the fuel) and oxidant (air or nitrous oxide) and by doing so, creates a negative pressure at the end of the small diameter, plastic nebulizer tube.
- This negative pressure acts to suck (uptake) liquid samples up the tube and into the nebulizer chamber, a process called **aspiration**.
- A small glass impact bead and / or a fixed impeller inside the chamber create a heterogeneous mixer of gas (fuel + oxidant) and suspended aerosol (finely dispersed sample).
- This mixture flows immediately into the burner head where it burns a smooth, laminar flame evenly distributed along a narrow slot in the well-machined metal burner.
- Liquid sample not flowing into the flame collects on the bottom of the nebulizer chamber and flows by gravity through a waste tube to a glass waste container.
- For some elements that form refractory oxides (molecules hard to break down in the flame) nitrous oxide (N_2O) needs to be used instead of air (78% N_2 + 21% O_2) for the oxidant.
- In that case, a slightly different burner head with a shorter burner slot length is used.

Nebulizer:

- In nebulization sample is converted in to a fine mist or droplets using a jet of compressed gas.
- The flow carries the sample into the atomization region.

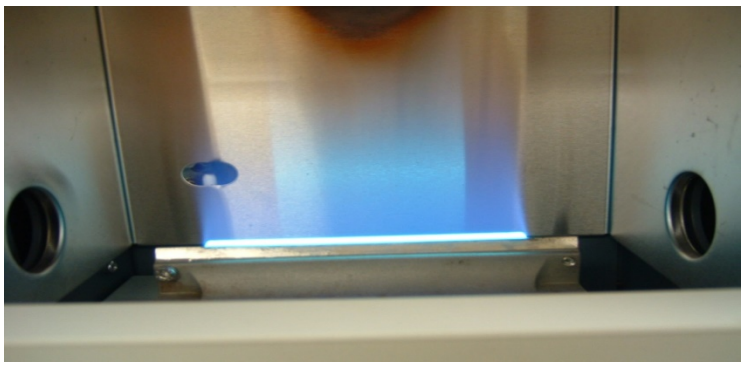
Pneumatic Nebulizers:

The liquid sample to be analyzed is sucked by a capillary with high pressure by a gas moving at high velocity. This is process is also called as aspiration.

Because of high velocity sample breaks into a fine droplets (mist) and carries it to the atomization region.

Laminar Flow:

- The mixture of gases and sample is directed into the burner head and flame.
- The burner is a specially one for AAS.
- It has a long, flat topped head positioned directly below and parallel to the beam of light from the lamp.
- The gases flow through a 10cm long slot at the top of the burner head so that a long, thin curtain of flame is produced.
- The two holes, left and right, are where the light beam enters and leaves after passing through the flame.
- The dark place at the top is a stain from the heat of the flame.



- The light from the hollow cathode lamp passes through the full 10cm length of the flame, greatly enhancing the absorption of light by the ground state atoms in the flame.
- The narrowness of the slot also concentrates the atoms and results in greater efficiency of light absorption.
- Burner head is made up of solid titanium which is corrosion resistant and free of most of the elements commonly determined by absorption.

4. Automizer:

In the automizer the sample solution is vapourised and the molecules are atomised.

Atomiser can be of two types.

1) Flow atomiser: Laminar consumption or total flow
2) Electro-thermal atomiser or graphite furnace

The flame is usually in the form of a sheet to increase path length and hence increase the absorbance and sensitivity.

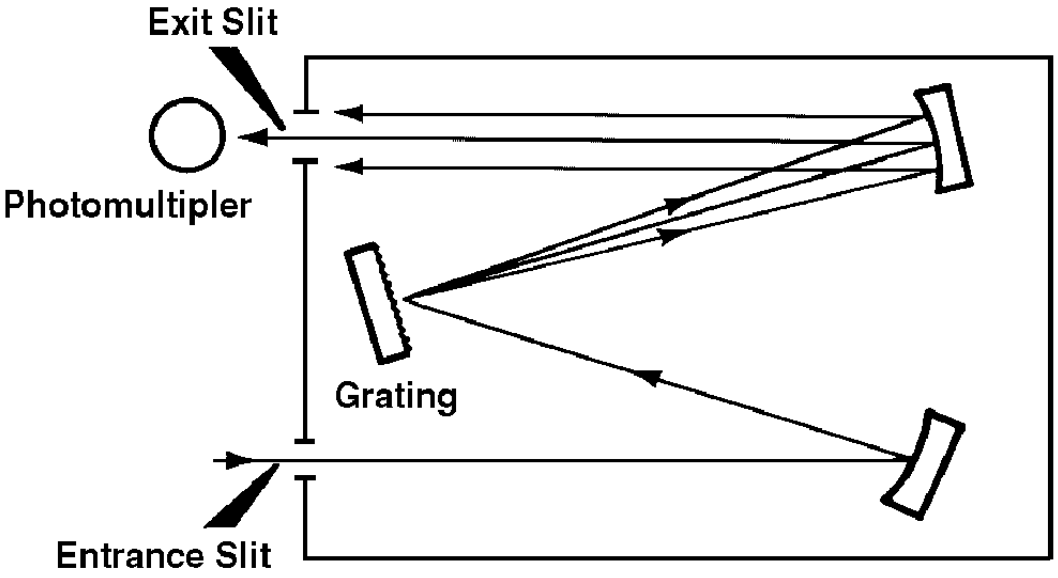
In electro-thermal atomiser, graphite rods heated by passing current and then sample goes through three phases to get vaporised. Drying-the solvent is evaporated, pyrolysis, sample temperature is then increased rapidly to vaporise it. Light is then passed through the sample.

5. Monochromator:

Monochromators are the devices that can selectively provide radiation of desired wavelength out of the range of wavelengths emitted by the source (lamp) or emitted by the analyte sample. Commonly used monochromators in AAS are prisms and gratings. Monochromator select monochromatic light from polychromatic light emitted by a hallow cathode lamp.

In AAS, the monochromator selects a given emission line and isolate it from other lines.

- Light from the source (i.e., flame) enters the monochromotor at the entrance slit and is directed to the grating where dispersion takes place.
- The diverging wavelength of the light are directed toward the exit slit.
- By adjusting the angle of the grating, a selected emission line from the source can be allowed to pass through the exit slit and fall onto the detector.
- All other lines are blocked from exiting.



6. Detectors:

This part of the instrument detects the intensity of radiation absorbed by the elements. The detector consists of a photomultiplier tube or simple photocell. The current or potential recorded for the sample absorption is recorded in computer software and then analyzed.

7. Amplifier:

It amplify the current from photomultiplier tube**.**

8. Read Out Device:

Chart recorder is the most common read out device. Micro ammeter is the other read out device used in AAS.

15.5. ADVANTAGES OF AAS:

- Solutions, slurries and solid samples can be analysed
- Much more efficient atomization
- Greater sensitivity
- Smaller quantities of sample (typically 5-5-µl)
- Provides a reducing environment for easily oxidised elements

15.6. DISADVANTAGES OF AAS:

- Expensive
- Low precision
- Low sample throughput
- Requires high level of skill operator
- Individual source lamps required for each element

15.7. LIMITATIONS OF AAS:

- Cannot detect non-metals
- New equipment is quite expensive
- More geared towards analysis of liquids
- Sample is destroyed

15.8. APPLICATIONS OF AAS:

Atomic spectroscopy is used for quantitative analysis of metal elements in water, soil, plant material and ceramics.

- **Mining and Geology**-The elemental composition of minerals and rocks provide valuable information on the commercial feasibility of conducting mining activities in areas explored.
- Trace metal analysis is of great value in prospecting for oil and water deposits.
- **Environmental Monitoring**–for trace metal contamination of industrial effluents, oceans, rivers and lakes.
- **Pharmaceuticals–**Trace metal analysis plays an important role in formulation development, catalyst efficiency and dosage limits.

- **Foods and Beverages**–In synthetic processed foods, metal pick up takes place due to contact with processing equipment and catalytic conversions. Manufacturer shave to ensure that the trace metals do not exceed the permissible limits and this requires vigorous quality control through atomic absorption spectroscopy and other sophisticated instruments.
- **Oil and Petroleum**–Both edible oils and mineral oils require refining before consumption.
- Uptake of metals during such operations can lead to deterioration of performance or consumer hazards.
- Trace metal analysis of engine oil provides useful diagnostic information on the wear and tear of engine parts.
- **Agriculture**–Trace metal constitution of soils in addition to the air acidic or basic nature is essential to establish their productivity and nutrient value.
- Trace metal composition of plants (leaves, stems and roots) gives a fair idea on how the uptake of minerals gets distributed under different growth conditions
- **Forensics–**Trace metal analysis provides valuable information on specimens such as stomach contents for food poisoning, paint chips, fibres and hair strands collected from the scene of a crime.

Others:

- In health care, it is used to analyze ionic metal elements in blood, saliva, urine samples. The elements analyzed routinely include sodium, potassium, magnesium, calcium and zinc.
- To determine heavy metals like iron, manganese, copper, zinc, mercury, lead, nickel, and in urine and blood.
- This analysis is essential in case of heavy metal poisoning. Since heavy metal poisoning is mostly lethal a regular monitoring of poison levels in the patient blood are essential.
- To determine metal elements like copper, nickel and zinc in the food industry.
- To estimate Pb, Ba, Ca, Na, Li, Zn and Mg in petroleum products.
- To determine metal concentrations (Ca, Mg, Fe, Si, Al, Ba content) in groundwater and bore well samplings before using for drinking and irrigation.
- Analysis of additives in lubricating oils and greases
- Clinical analysis (blood samples, plasma serum – Ca, Mg, Li, Na, K and Fe).

15.9. SUMMARY:

- To know about the Atomic Absorption Spectroscopy.
- To study about Principle involved in AAS.
- To study about Instrumentation of AAS.
- To study about Advantages, Disadvantages and Applications of AAS.

15.10. SELF-ASSESSMENT QUESTIONS:

1) Write the basic principle involved in AAS.
2) Write the principle and instrumentation of AAS.
3) Write instrumentation, Advantages and limitations of AAS.
4) Define AAS? Write the applications of AAS.

15.11. REFERENCE BOOKS:

1) Introduction to Organic Laboratory Techniques-D.L. Pavia, G.M. Lampman, G. S. Kriz and R.G. Engel, Saunders College Pub. (NY).
2) Instrumental Methods of Chemical Analysis by B.K. Sharma, Goel Publish House, Meerut.
3) Instrumental Methods of Chemical Analysis by H. Kaur, Pragati Prakasan, Meerut.
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5) Principles of Instrumental Analysis by D.A. Skoog, F.J. Holler and T.A. Nieman, Harcourt College Pub.

Dr. K. Chandra Mohan

LESSON-16

INDUCTIVE COUPLED PLASMA-OPTIMISED EMISSION SPECTROSCOPY (ICP-OES)

16.0. OBJECTIVES:

After studying this lesson, you should be able to:

- To know about the ICP-OES/AES.
- To study about basic Principle involved in ICP-OES/AES.
- To study about Instrumentation of ICP-OES/AES.
- To study about Advantages, Disadvantages and Applications of ICP-OES/AES.
- To learn about advantages of ICP-AES over than AAS.

STRUCTURE:

16.1 Introduction

16.2 Basic Principle

16.3 Instrumentation of ICP-OES/AES

16.4 Applications of ICP-AES

16.5 Advantages of ICP-AES over than AAS

16.6 Summary

16.7 Self-Assessment Questions

16.8 Reference Books

16.1. INTRODUCTION:

ICP-OES is a sophisticated instrument used in determination of trace concentrations of elements in sample based on atomic spectrometry, after due pretreatment.

Inductively Coupling:

Process of transferring energy to a system through the use of electromotive forces generated by magnetic fields.

Plasma:

A state of matter usually consisting of highly ionized gas that contains an appreciable fraction of equal numbers of ions and electrons in addition to neutral atoms and molecules.

Inductively Coupled Plasma (ICP):

A high temperature discharge generated by flowing a conductive gas through a magnetic field induced by a load coil that surrounds the tubes carrying the gas.

Optical Emission Spectrometry (OES):

Elemental analysis technique that uses emission of electromagnetic radiation to detect the presence of the elements of interest.

Importance of ICP-AES:

- ICP-OES can handle geological, mining, and rare earth elements.
- ICP-OES is widely used in mining processes, mining purity control, rocks analysis, etc.
- Many mines use ICP-OES to check for the purity of the extracted ores of manganese, nickel or precious metals.

16.2. BASIC PRINCIPLE:

A sample aerosol is generated in an appropriate nebulizer and spray chamber and is carried into the plasma through an injector tube located within the torch. Due to high temperature at Plasma region, the sample undergoes desolvation, vaporization, atomization, excitation and ionization. The Normal analytical zone is the region of the Plasma from which analyte emission is measured. From the wavelength, the element is identified. From the emission count, concentration of the analyte is determined.

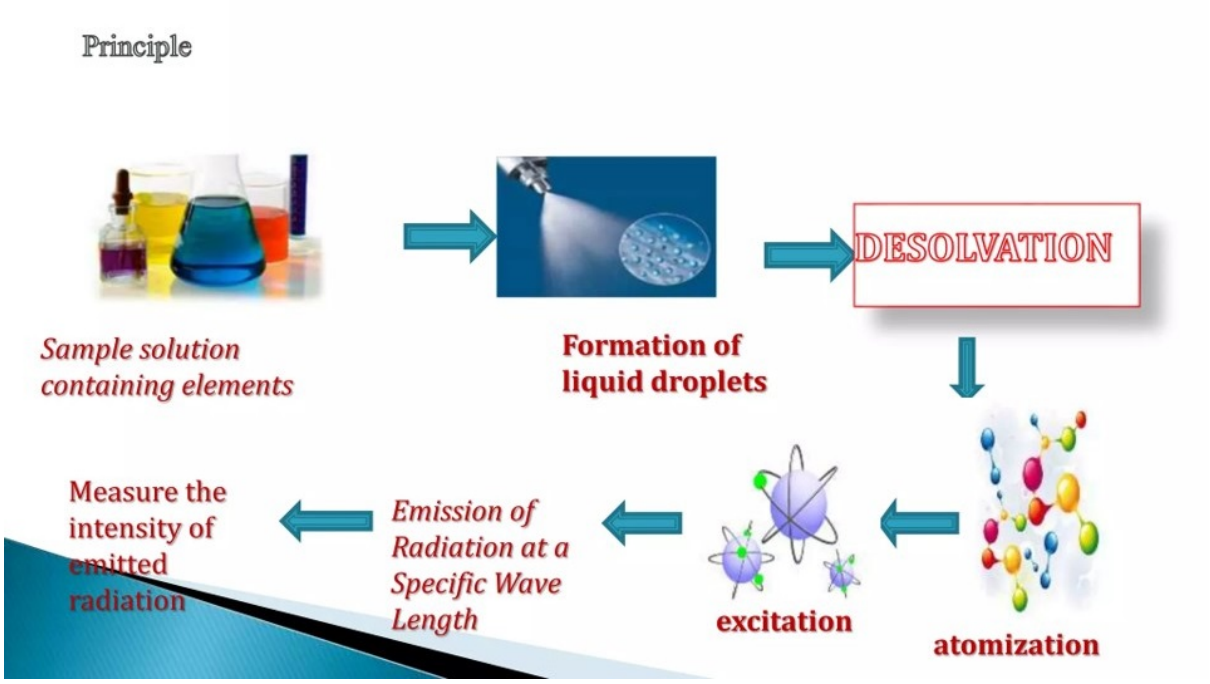

Figure 1: Process Takes Place at ICP Discharge

16.3. INSTRUMENTATION OF ICP-OES/AES:

Components of ICP:

1. Sample Introduction System:

A) Peristaltic Pump, B) Nebulizer, C) Spray Chambers, D) Drains

2.Plasma:

A) Demountable ICP torch B) RF Generators

3.Spectrometer:

A) Slit, B) Collimator, C) Gratings, D) Prism, E) Photo Multiplier Tube

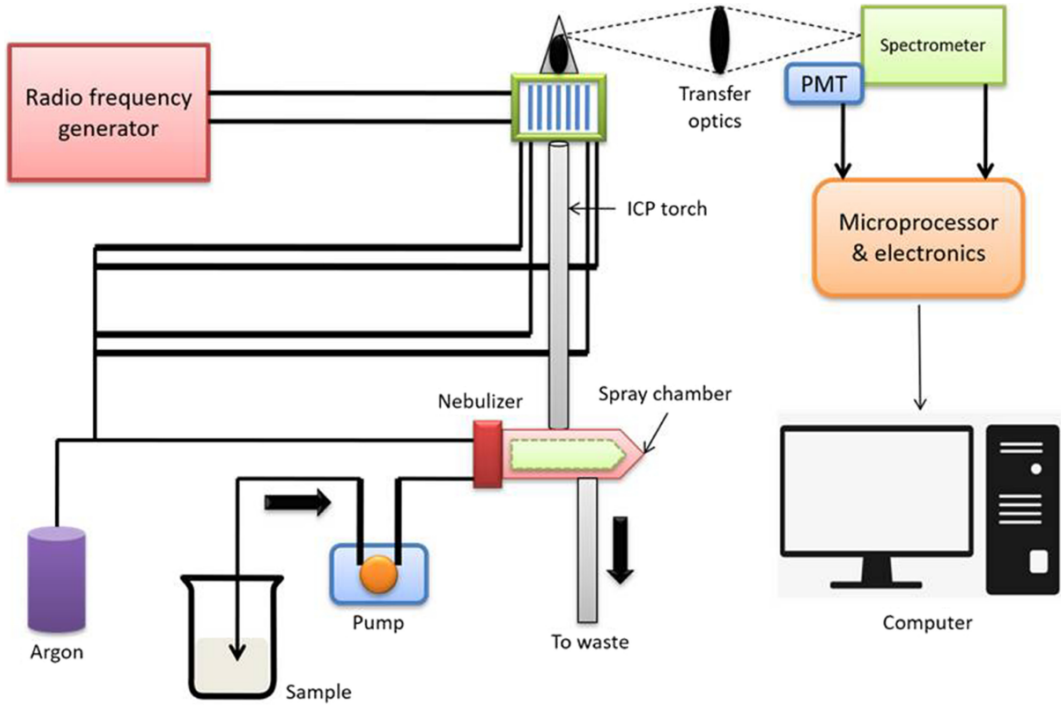

Figure 2: Schematic Representation of ICP-AES

1. Sample Introduction System:

A. Peristaltic Pump:

A pump in which the fluid is pushed through a length of flexible tubing by waves of mechanical contractions, usually caused by a series of rollers that travel along the length of the tubing. So, the flow is laminar without any pulse.

B. Nebulizer:

A device used to create an aerosol from a liquid. A high-speed stream of argon gas is directed perpendicular to the tip of a capillary tube. The solution is either drawn up through the capillary tube by the low-pressure region created by the high-speed gas or forced up the tube with a pump. In either case, contact between the high-speed gas and the liquid stream causes the liquid to break up into an aerosol.

C. Spray Chamber:

A device placed between a nebulizer and an atomization/excitation source, whose function is to separate the aerosol droplets according to their size and to smooth out fluctuations in the sample carrying gas flow.

The primary function of spray chamber is to remove large droplets from the aerosol. A secondary purpose is to smooth out pulses that occur during nebulization, often due to pumping of the solution. For ICP operation, it is designed to allow droplets with diameter of about 10 microns or smaller to pass on to the plasma.

D. Drains (Waste):

Drain carries excess sample from the spray chamber to a waste container can have an impact on the performance of ICP. It also provides the backpressure necessary to force the sample aerosol carrying nebulizer gas flow through the torch's injector tube and into the plasma discharge.

2. Plasma:

A. Torches:

The torch contains three concentric tubes. The spacing between the two outer tubes is kept narrow so that the gas introduced between them emerges at high velocity. This outside chamber is also designed to make the gas spiral tangentially around the chamber as it proceeds upward and acts as a coolant (PLASMA FLOW). The chamber between the outer flow and the inner flow sends gas directly under the plasma toroid. This flow is called (INTERMEDIATE FLOW) and prevents carbon formation on the tip of the injector tube.

Nebulizer Flow:

The gas flow that carries the sample aerosol is injected into the plasma through the central tube or injector. Due to small diameter at the end of the injector, the nebulizer flow can punch a hole through the plasma.

B. Radio Frequency Generators:

The device that provides the power for the generation and sustainment of the plasma discharge. This power, typically ranging from about 700 – 1500 watts, is transferred to the plasma gas through a load coil surrounding the top of the torch.

3. Spectrometer:

Photomultiplier Tube/Detector:

Photo multiplier tube or PMT is a vacuum tube that contains a photosensitive material called the photocathode, that ejects electrons when it is struck by light. These electrons are accelerated towards a dynode which ejects two to five secondary electrons strike another dynode, ejecting more electrons which strike yet another dynode, causing a multiplicative effect along the way. Typical PMTs contain 9 to 16 stages. Collection of secondary electrons from the last dynode by the anode. The electrical current measured at the anode is then used as a relative measure of the intensity of the radiation reaching the PMT.

16.4. APPLICATIONS OF ICP-AES:

- **Clinical analysis**: melas in biological fluids (blood, urine).
- **Environmental analysis**: Trace metals and other elements in water, air, soil, plants, compost, and sludges.

- **Pharmaceutical analysis**: trace of catalysts used; traces of poison metals like Cd, Pb, etc.
- **Industry**: Trace metal analysis in raw materials, Nobel metal determination.
- **Forensic science**: Gunshot powder residue analysis, toxicological examination (Tl, Hg etc.)

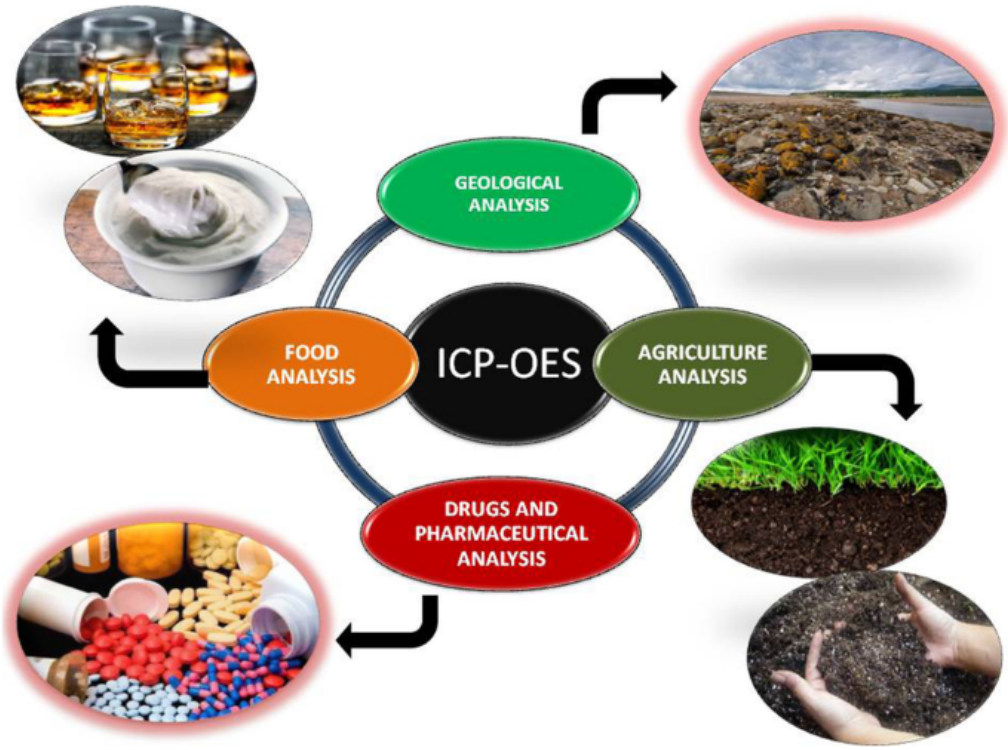

Figure 3: Schematic Representation for Applications of ICP-AES/OES

The application of ICP-AES includes:

- The determination of metals in wine, arsenic in food, and trace elements bound to proteins. ICP-AES methods are used to test for metals contamination in drinking water and wastewater.
- ICP-AES is widely used in minerals processing to provide the data on grades of various streams, for the construction of mass balances.
- ICP-AES is often used for analysis of trace elements in soil, and it is for that reason it is often used in forensics to ascertain the origin of soil samples found at crime scenes or on victims etc
- ICP-AES is used for motor oil analysis. Analysing used motor oil reveals a great deal about how the engine is operating. Parts that wear in the engine will deposit traces in the oil which can be detected with ICP-AES. ICP-AES analysis can help to determine whether parts are failing. ICP-AES is also used during the production of motor oils (and other lubricating oils) for quality control and compliance with production and industry specifications.

16.5. ADVANTAGES OF ICP-AES OVER THAN AAS:

ICEP-AES/OES	AAS
Emission	**Absorption**
Is the process that creates a photon and takes the atom or molecular in an excited state back to the ground state.	Is the process that consumes a photon and puts the atom or molecule in an excited state.
Many interferences are used	Few interferences are used
All elements in the sample can be analyzed in the same time, multi elements analysis.	Elements by element, single element analysis.
Prepare 3-5 points for Calibration for all the required elements	Prepare 3-5 points for Calibration for each element
Short time for standards preparation	Long time for standards preparation
Consume less glass ware	Consume a lot of glass ware
One report for all results	One report for each element

16.6. SUMMARY:

- To know about the ICP-OES/AES.
- To study about basic Principle involved in ICP-OES/AES.
- To study about Instrumentation of ICP-OES/AES.
- To study about Advantages, Disadvantages and Applications of ICP-OES/AES.
- To learn about advantages of ICP-AES over than AAS.

16.7. SELF-ASSESSMENT QUESTIONS:

1) Write the basic principle involved in ICP-AES.
2) Write the principle and instrumentation of ICP-AES.
3) Write instrumentation, Advantages and limitations of ICP-AES.
4) Define ICP-AES? Write the applications of ICP-AES.
5) Write the advantages of ICP-AES over than AAS.

16.8. REFERENCE BOOKS:

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Dr. K. Chandra Mohan

LESSON-17

ULTRA-VIOLET AND VISIBLE SPECTROSCOPY

17.0. OBJECTIVES:

After studying this lesson, you should be able to:

- To know about the UV-visible Spectroscopy.
- To study about basic Principle involved in UV-visible Spectroscopy.
- To learn about effect of solvent on electronic transitions in UV-visible Spectroscopy.
- To study about Instrumentation of UV-visible Spectroscopy.
- To study about Advantages, Disadvantages and Applications of UV-visible Spectroscopy.

STRUCTURE:

17.1 Introduction

17.2 Basic Principle

17.3 Spectrophotometer and Spectrum Recording

17.4 Process of Electronic Excitation

17.5 Electronic Transitions

17.6 Effect of Solvent on Electronic Transitions

17.7 UV-Visible spectrophotometer Instrumentation

17.8 Applications of UV-Visible Spectroscopy

17.9 Disadvantages of UV-Vis Spectroscopy

17.10 Summary

17.11 Self-Assessment Questions

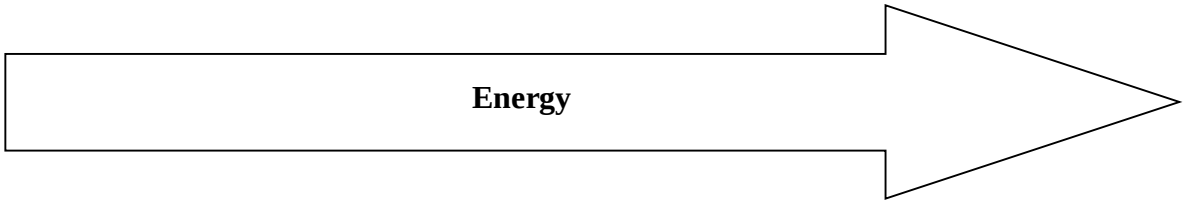
17.12 Reference Books

17.1. INTRODUCTION:

Most organic molecules and functional groups are transparent in the portions of the electromagnetic spectrum that we call the ultraviolet (UV) and visible (VIS) regions-that is, the regions where wavelengths range from 190 nm to 800 nm. Consequently, absorption spectroscopy is of limited utility in this range of wavelengths. However, in some cases we can derive useful information from these regions of the spectrum. That information, when combined with the details provided by infrared and nuclear magnetic resonance (NMR) spectra, can lead to valuable structural proposals.

17.2. BASIC PRINCIPLE:

Ultra violet-Visible spectroscopy is primarily used to understand the structure of compounds having multiple bonds or aromatic conjugation within the molecules. The useful UV region starts from 200 - 400 nm and the visible region from 400- 800mm.



800-400 nm **400-200nm** **190-100nm** **Soft X-rays**

Visible **Near UV** **Vacuum UV**

When the electromagnetic radiation in UV-Visible region passes through a compound with multiple bonds, a portion of the radiation is normally absorbed by the compound.

The absorption depends on the wavelength of the radiation and on the structure of the compound. The electrons in orbitals of lower energy are excited in to orbitals of high energy by the absorption of radiation. Generally, the electrons are excited from HOMO to LUMO in organic molecules. Since the electrons are excited by radiation, UV- Visible spectroscopy is also called as electronic spectroscopy. UV-Visible spectrum records a plot of absorbance verses wavelength. The extent of absorption is given as absorbance A, or transmittance T or ε (molar extinction coefficient also known as molar absorptivity, a measure of extent of absorption) or log ε. The molar absorptivity as defined by the Beer Lambert's law gives the relationship between ε and absorption (**Figure 17.1**).

$$\log\frac{I_o}{I} = \varepsilon c l \text{ or } A = \varepsilon c l$$

Where I_o = the intensity of the incident radiation,

I = the intensity of the transmitted radiation

A = Absorbance of the solution (optical density OD)

c= Concentration of the solution gram moles/lit (density OD) letter

l = Path length of the sample (in cm),

ε = Molar absorptivity (extinction coefficient)

$T = I/I_o$ is the transmittance

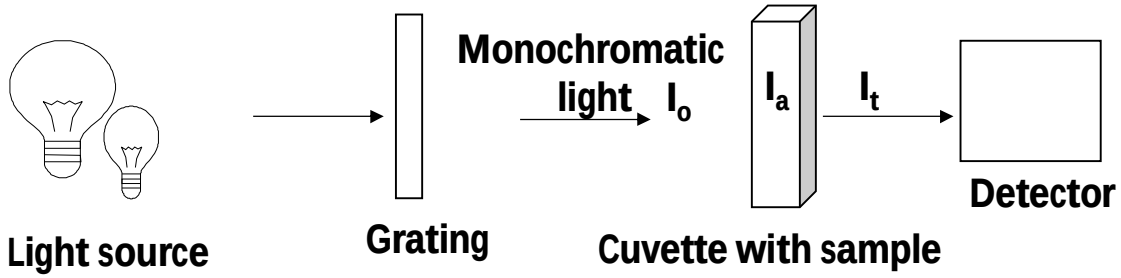


Figure 17.1: The process of Absorption of Light Radiation in a UV-Visible Spectroscopy.

If the concentration (c) of the solute is now defined as grams per liter then the equation becomes.

$$A = a\ cl$$

Where 'a' is the absorptivity and thus related to the molar absorptivity

$$\varepsilon = a\ M$$

Where M is the molecular weight of the solute.

The maximum point of absorption band is indicated as λ_{max}, which means that the position where maximum absorption, ε_{max}, the molar extinction coefficient is the intensity of the band at the maximum wavelength.

17.3. SPECTROPHOTOMETER AND SPECTRUM RECORDING:

UV radiation can be generated from hydrogen/deuterium tube, while visible light can be obtained from tungsten filament. When the light (Polychromatic) is passed through the prism it is diffracted to monochromatic light which is selectively passed through a mirror, split in to two parallel beams one passing through the solution containing the sample in appropriate solvent and other through the reference sample (pure solvent). The intensity of the transmitted beams of reference and sample are compared over the whole range of wave length an disdetected through photoelectric tubes generating alternating currents, which are then recorded **(Figure 17.2**).

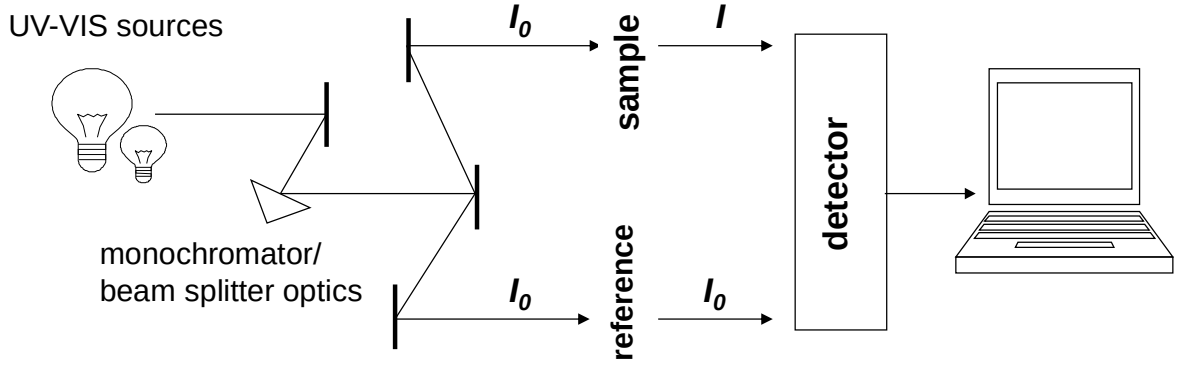
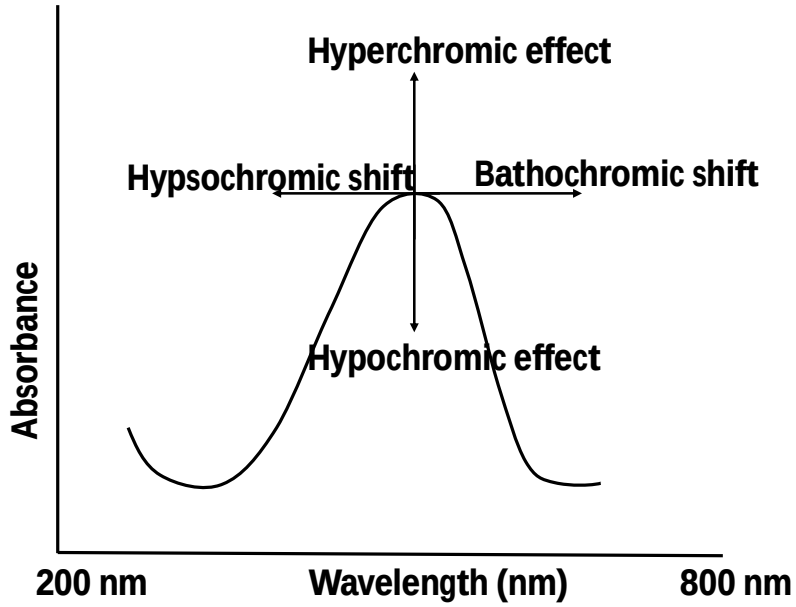


Figure 17.2: The Double Beam UV-Visible Spectrophotometer

Absorption bands of an UV-Visible spectra are typically broad when compared to IR and NMR spectra. This is due to the fact that a large number of different electronic transitions occur between the several different allowed vibrations and rotational energy levels. Though these transitions are quantized, the energy differences are very close and hence a broad band is noticed.

It is observed that only certain functional groups present in the molecule is responsible for the absorption of the light in the UV region or in the visible region and such groups are known as chromophores. Chromophore is an isolated functional group capable of absorbing visible and or UV radiation. Some of the typical chromophores are C=C, C= N, N=N, C=O, etc. There are certain other groups which are not directly responsible for the absorption of radiation, but are capable of influencing the position and intensity of absorption band and these groups are known as auxochromes. An auxochrome is a functional group which does not absorb radiation but when attached to a given chromophore causes a shift in the absorption to alonger and shorter wavelength and also increases the degree of absorption. -NH_2, -NHR, -OH, -OR, -CH_3 etc. are good auxochromes. These substituents may have the following four kinds of effects on the absorption.

1. **Bathochromic (Red) Shift**-A shift to longer wavelength or lower energy
2. **Hypsochromic (Blue) Shift** -A shift to a shorter wavelength or higher energy
3. **Hyperchromic Effect**- An increase in intensity of absorption
4. **Hypochromic Effect**– A decrease in intensity of absorption



17.4. PROCESS OF ELECTRONIC EXCITATION:

The bond formation between two atoms involves the overlap of two atomic orbitals each containing one electron leading to new molecular orbitals **(Figure 17.3**). One of them with lower in energy is bonding molecular orbital and the other with higher energy is known as antibonding molecular orbital. The bonding molecular orbital is filled with two paired

electrons and the antibonding is supposed to be vacant. Some molecules have non-bonding orbitals with valence electrons. On absorption of energy by a molecule in the UV-Visible region, changes are produced in the electronic energy of the molecule due to transitions of valence electrons in the molecule. These transitions consist of the excitation of an electron from an occupied molecular orbital (HOMO) to next higher energy antibonding orbital (LUMO). The antibonding orbitalis indicated by an asterisk. The promotion of an electron from a π bonding orbital to an antibonding π^*orbital is designated as $\pi \rightarrow \pi^*$ similarly $n \rightarrow \pi^*$, $\pi \rightarrow \sigma^*$.

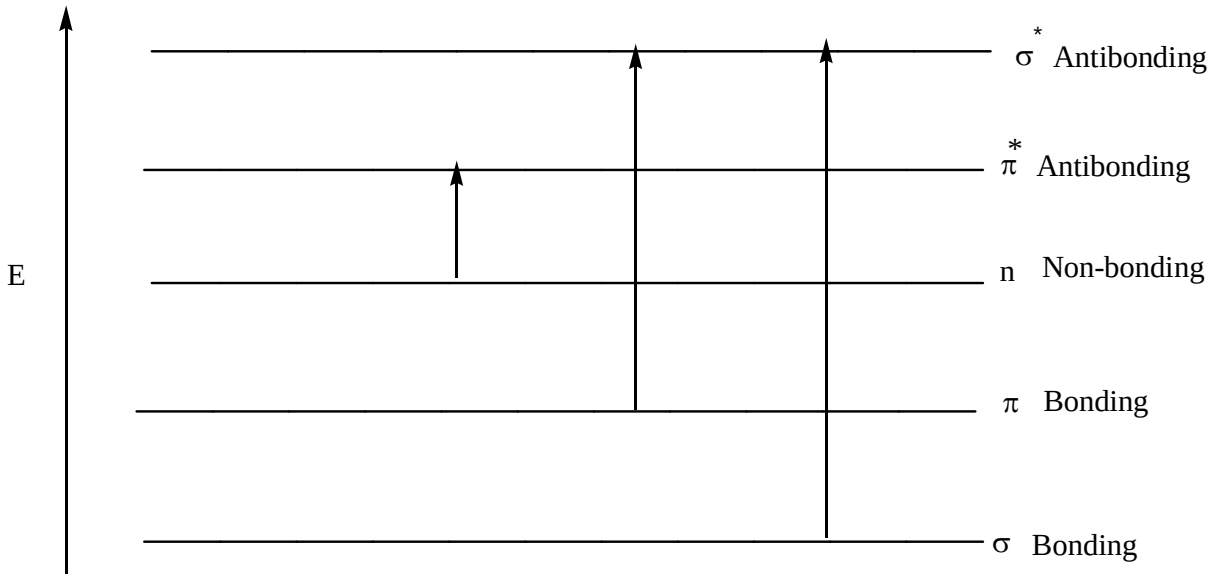


Figure 17.3: Energy Levels of Bonding, Nonbonding and Antibonding Orbitals

17.5. ELECTRONIC TRANSITIONS:

The relative energies of the more important orbitals are shown as above.

i) $\sigma \rightarrow \sigma^*$ Transition:

The transition of an electron from a bonding sigma orbital to the higher energy antibonding sigma orbital is designated as $\sigma \rightarrow \sigma^*$and it is found in alkanes. Sigma bonds are in general strong; therefore, it is a high energy process **(Figure 17.4)** and these transitions require very short wavelengths and high energy, such transitions occur in vacuum UV region. i.e. below 200 nm, not in the UV-Visible spectroscopic range.

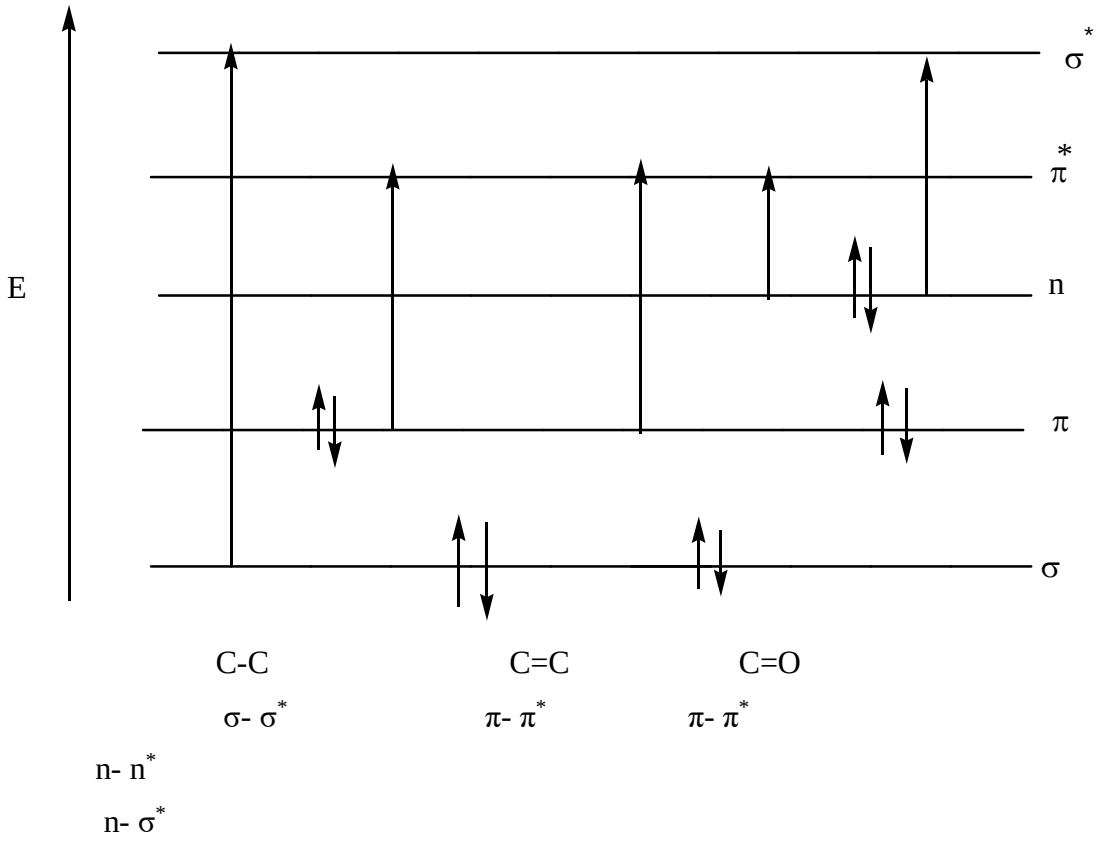


Figure 17.4: Various Transition Involved in UV Spectroscopy

ii) $n \rightarrow \sigma^*$ Transition:

It involves saturated compounds with one hetero atom having unshared pair of electrons, Ex. Saturated halides, alcohols, ethers, aldehydes, ketones, amines etc. They require less energy than $\sigma \rightarrow \sigma^*$ transitions. Water absorbat167; methylalcohol at 174, methylchloride at 169 and methyl iodide at 258 nm. In saturated alkyl halides, the energy required for the transition decreases with the increase in the size of the halogen atom. Due to the more electro negativity of chlorine than iodine, the non-bonding electrons of chlorine atoms are comparatively difficult to excite. Therefore, the transition is easier in the case of methyliodide and its molecular extinction coefficient is also higher than methylchloride.

iii) $\pi \rightarrow \pi^*$ Transition:

This type is found in the compounds with unsaturated centers like simple alkenes, aromatics, carbonyl compounds, etc. This transition requires less energy than $n \rightarrow \sigma^*$ transition, this lowest energy transition absorbs around 170-190 nm in unconjugated alkenes. In the saturated ketoses the most intense bond occurs around 150 nm due to $\pi \rightarrow \pi^*$ transition.

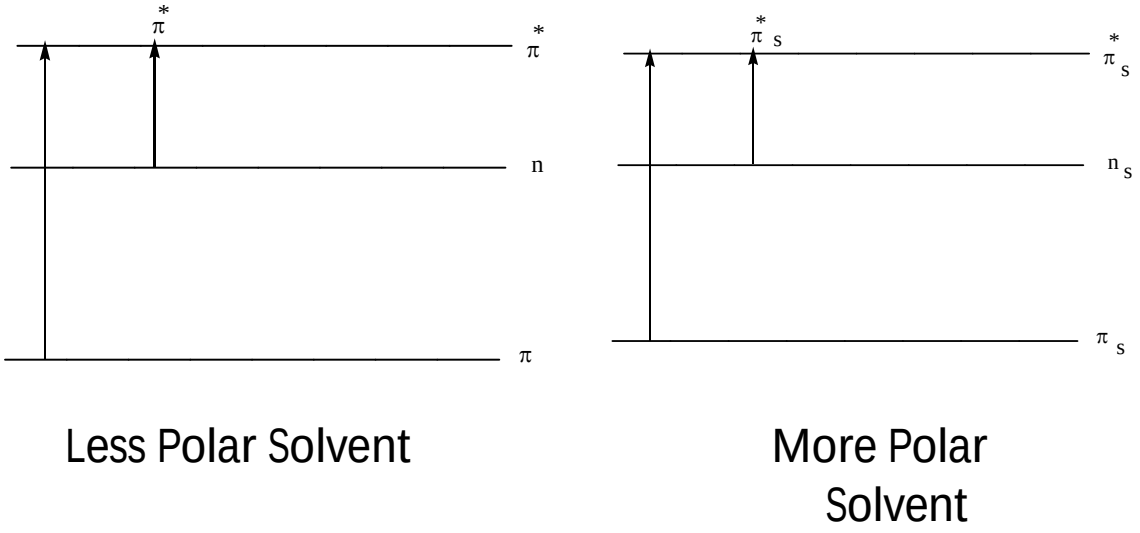
iv) $n \rightarrow \pi^*$ Transition:

In this the electron of unshared electron pair on a hetero atom is excited to π^* antibonding orbital. It involves least amount of energy than all the transitions and so it gives

rise to an absorption band at longer wavelengths. The $n \rightarrow \pi^*$ transition in saturated aliphatic ketones is around 280 nm is the lowest energy transition. On the other hand the $n \rightarrow \pi^*$ transition is a forbidden transition because the non-bonding orbital is orthogonal to π^*orbital with a poor overlap integral. Hence it leads to a very weak absorption, ε value is less than1000.

17.6. EFFECT OF SOLVENT ON ELECTRONIC TRANSITIONS:

The solvents have absorption and they must be transparent with in the wavelength range being examined. The position and intensity of absorption b and may shift with the change in this polarity solvent in which it is being recorded (**Figure 17.5**). The transitions of polar bonds like C=O, C=N are affected by solvent polarity. As this solvent polarity increases $\pi \rightarrow \pi^*$ bands undergo red shifts (longer wavelength) while $n \rightarrow \pi^*$ bands move to lower wavelength region. A hypsochromic shift is observed in polar solvents if the ground state is relatively more polar than its structure in the excited state. In the other way if the excited state is more polar than the ground state, a bathochromic shift is observed. The polar solvents stabilize the π, $\pi^*$and n orbitals by salvation. The stabilization of n orbital is particularly pronounced with this by dragon. Bonding solvents and π^* is mole stabilized than π. This is because of this fact that π^* orbital is more polar.



Solvent effect π to π* >πs>π*s n toπ*< ns to πs

Figure 17.5: Absorption on Shift with Change in Polarity of the Solvent

The energy of transitions for $\pi \rightarrow \pi^*$becomes less with solvation effecting red shift, while the energy of transition for $n \rightarrow \pi^*$ becomes greater with solvation leading to blue shift when the solvent is changed from ethanol to other.

The shift for a given solvent is as follows

Solvent	Wavelength (nm)
Water	+8 nm
Methanol	0 nm
Chloroform	-1 nm
Dioxane	-5 nm
Diethyl ethyl	-7 nm
Hexane	-11 nm
Cyclohexane	-11 nm

17.7. UV-VISIBLE SPECTROPHOTOMETER INSTRUMENTATION:

The typical ultraviolet–visible spectrophotometer consists of a light source, a monochromator, and a detector (Figure 17.6**)**

Light Source:

- Tungsten filament lamps and Hydrogen-Deuterium lamps are the most widely used and suitable light sources as they cover the whole UV region.
- Tungsten filament lamps are rich in red radiations; more specifically they emit the radiations of 375 nm, while the intensity of Hydrogen-Deuterium lamps falls below 375 nm.

Monochromator:

- Monochromators generally are composed of prisms and slits.
- Most of the spectrophotometers are double beam spectrophotometers.
- The radiation emitted from the primary source is dispersed with the help of rotating prisms.
- The various wavelengths of the light source which are separated by the prism are then selected by the slits such the rotation of the prism results in a series of continuously increasing wavelengths to pass through the slits for recording purposes.
- The beam selected by the slit is monochromatic and further divided into two beams with the help of another prism.

Sample and Reference Cells:

- One of the two divided beams is passed through the sample solution and the second beam is passé through the reference solution.
- Both sample and reference solution is contained in the cells.

- These cells are made of either silica or quartz. Glass can't be used for the cells as it also absorbs light in the UV region.

Detector:

- Generally, two photocells serve the purpose of the detector in UV spectroscopy.
- One of the photocells receives the beam from the sample cell and the second detector receives the beam from the reference.
- The intensity of the radiation from the reference cell is stronger than the beam of the sample cell. This results in the generation of pulsating or alternating currents in the photocells.

Amplifier:

- The alternating current generated in the photocells is transferred to the amplifier.
- The amplifier is coupled to a small servometer.
- Generally, the current generated in the photocells is of very low intensity, the main purpose of the amplifier is to amplify the signals many times so we can get clear and recordable signals.

Recording Devices:

- Most of the time amplifier is coupled to a pen recorder which is connected to the computer.
- The computer stores all the data generated and produces the spectrum of the desired compound.

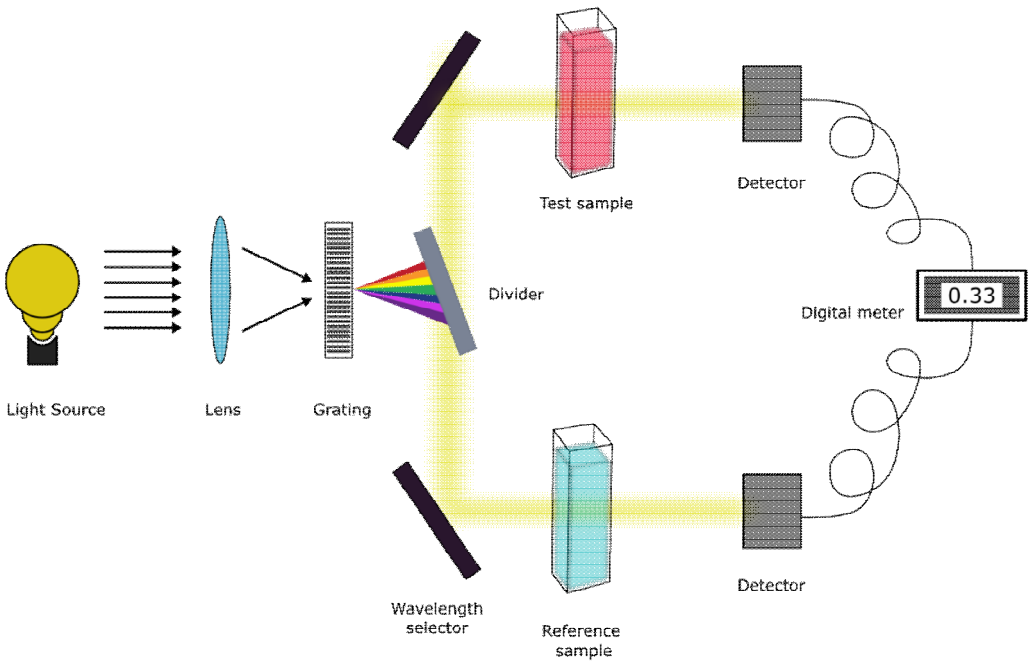


Figure 17.6: Double Beam UV Visible Spectrophotometer

17.8. APPLICATIONS OF UV-VISIBLE SPECTROSCOPY:

Detection of Impurities:

- It is one of the best methods for the determination of impurities in organic molecules.
- Additional peaks can be observed due to impurities in the sample and it can be compared with that of standard raw material.
- By also measuring the absorbance at a specific wavelength, the impurities can be detected.

Structure Elucidation of Organic Compounds:

It is useful in the structure elucidation of organic molecules, such as in detecting the presence or absence of unsaturation, the presence of heteroatoms.

1) UV absorption spectroscopy can be used for the **quantitative determination of compounds** that absorb UV radiation.
2) UV absorption spectroscopy can characterize those types of compounds that absorb UV radiation thus used in the qualitative determination of compounds. Identification is done by comparing the absorption spectrum with the spectra of known compounds.
3) This technique is used to detect the presence or absence of a functional group in the compound. The absence of a band at a particular wavelength is regarded as evidence for the absence of particular group.
4) Kinetics of reaction can also be studied using UV spectroscopy. The UV radiation is passed through the reaction cell and the absorbance changes can be observed.
5) Many drugs are either in the form of raw material or in the form of the formulation. They can be assayed by making a suitable solution of the drug in a solvent and measuring the absorbance at a specific wavelength.
6) Molecular weights of compounds can be measured spectrophotometrically by preparing the suitable derivatives of these compounds.
7) UV spectrophotometer may be used as a detector for HPLC.

17.9. DISADVANTAGES OF UV-VIS SPECTROSCOPY:

1) It may take time to prepare using the machine.
2) Spectrometer reading might be affected if it keeps with any electronic noise, outside light, and other contaminants.
3) The accuracy of the machine's measurement could be impacted by stray light from defective equipment design because the linearity range and substance absorbency measuring are likely to be reduced by stray light.

17.10. SUMMARY:

- To know about the UV-visible Spectroscopy.
- To study about basic Principle involved in UV-visible Spectroscopy.
- To learn about effect of solvent on electronic transitions in UV-visible Spectroscopy.

- To study about Instrumentation of UV-visible Spectroscopy.
- To know about various electronic transitions involved in UV-visible Spectroscopy.
- To study about Advantages, Disadvantages and Applications of UV-visible Spectroscopy.

17.11. SELF-ASSESSMENT QUESTIONS:

1) Write the basic principle and applications of UV-visible Spectroscopy.
2) Discuss the important of solvent effect in UV-visible Spectroscopy.
3) Explain the instrumentation of UV-visible Spectroscopy.
4) Write the electronic transitions involved in UV-visible Spectroscopy.

17.12. REFERENCE BOOKS:

1) Introduction to Organic Laboratory Techniques-D.L. Pavia, G.M. Lampman, G.S. Kriz and R. G. Engel, Saunders College Pub. (NY).
2) Instrumental methods of Chemical Analysis by B.K. Sharma, Goel Publish House, Meerut.
3) Instrumental methods of Chemical Analysis by H. Kaur, Pragati Prakasan, Meerut.
4) A Hand Book of Instrumental Techniques for Analytical Chemistry- Ed-F. A. Settle, Prearson Edn.

Dr. K. Chandra Mohan

LESSON-18

INFRARED SPECTROSCOPY

18.0. OBJECTIVES:

After studying this lesson, you should be able to:

- To know about the Infrared Spectroscopy.
- To study about basic Principle involved in Infrared Spectroscopy.
- To study about Instrumentation of Infrared Spectroscopy.
- To learn about molecular vibrations in Infrared Spectroscopy.
- To know about importance of Finger print Infrared Spectroscopy.
- To study about Advantages, Disadvantages and Applications of Uv-visible Spectroscopy.
- To learn about frequency ranges of various functional groups using Infrared Spectroscopy.

STRUCTURE:

18.1 Introduction

18.2 Molecular Vibrations:

18.3 Instrumentation of Infrared spectroscopy

18.4 Finger print region:

18.5 General IR frequencies for different bonds

18.6 Applications of Infrared (IR) Spectroscopy

18.7 Limitations and Challenges of IR Spectroscopy

18.8 Summary

18.9 Self-Assessment Questions

18.10 Reference Books

18.1. INTRODUCTION:

The absorption of infrared radiation by an organic compound causes' the various bands in a molecule to vibrate (stretch and bend). Some of the frequencies are absorbed and some other are transmitted without being absorbed. If we plot the percentage transmittance or percentage absorbance against wave number or frequency, infrared spectrum is obtained. The most important region for organic chemist is 4000 - 650 cm^{-1}or 2.5 – 15μ. The absorption of IR radiation can be expressed in terms of wavelength or wavenumber. Generally, IR spectra of 'organic compounds are plotted as percentage transmittance against wave number. The relationship between wave length and wave number is as follows.

$$\text{Wavenumber} = \frac{1}{\text{Wavelength (in centimeters)}}$$

If the wave length is 2.5 μ=2.5 x 10^{-4}cm

$$\bar{\nu}$$

$$\text{Wavenumber, } = \frac{1}{2.5 \times 10^{-4}} = 4000 \text{ cm}^{-1}$$

The transitions of energy involved in infrared absorption are associated with vibrational changes within the molecule. The molecules are constantly undergoing vibrations at room temperature. Each bond in the molecule has its characteristic stretching and bending vibrations which are quantized and are capable of absorbing light of that frequency lying in the IR region. The stretching vibration of two atoms connected to a bond can be compared to the vibrations of two balls attached to a spring. It requires more energy to stretch than to bend it. Thus, stretching absorption of bond appears at higher frequency than the bending absorption of the same bond. For non-linear polyatomic molecules, there will be 3N-6 modes of vibration (3N-5 if linear) which include both stretching and bending vibrations.

Hooke's Law. Calculation of Vibrational Frequency:

The stretching frequency ν of a bond with reasonable accuracy can be calculated applying Hooke's law of simple harmonic motions.

The wave number is

$$\bar{\nu} = \frac{1}{2\pi c}\sqrt{\frac{K}{\mu}} \text{ cm}^{-1}$$

$$\bar{\nu} = 4.12\sqrt{\frac{K}{\mu}} \text{ cm}^{-1}$$

Where μ= reduced mass $= \frac{m1\ m2}{m1 + m2}$

ν is the frequency of absorption

K =force constant of the bond whose value is $5x10^5$, $10x10^5$, $15x10^5$dynes/cm for a single, double and triple bond respectively.

m_1 and m_2, are the masses of the atoms attached to the covalent bond.

From the equation it is clear that the vibrational frequency of a bond will increase when the bond strength increases and also when this reduced mass of the system decreases. Thus, greater the mass lower the frequency of absorption and carbonyl bond will have higher vibrational frequency than C-O bond and C-H will have higher frequency of absorption than C-O.

18.2. MOLECULAR VIBRATIONS:

In stretching vibration, the distance between two atoms increases or decreases, however the atoms remain in the same bond axis. The vibration occurs at higher frequency. In bending the distance between these atoms remain constant and position of these atoms may change (**Figure 18.1**).

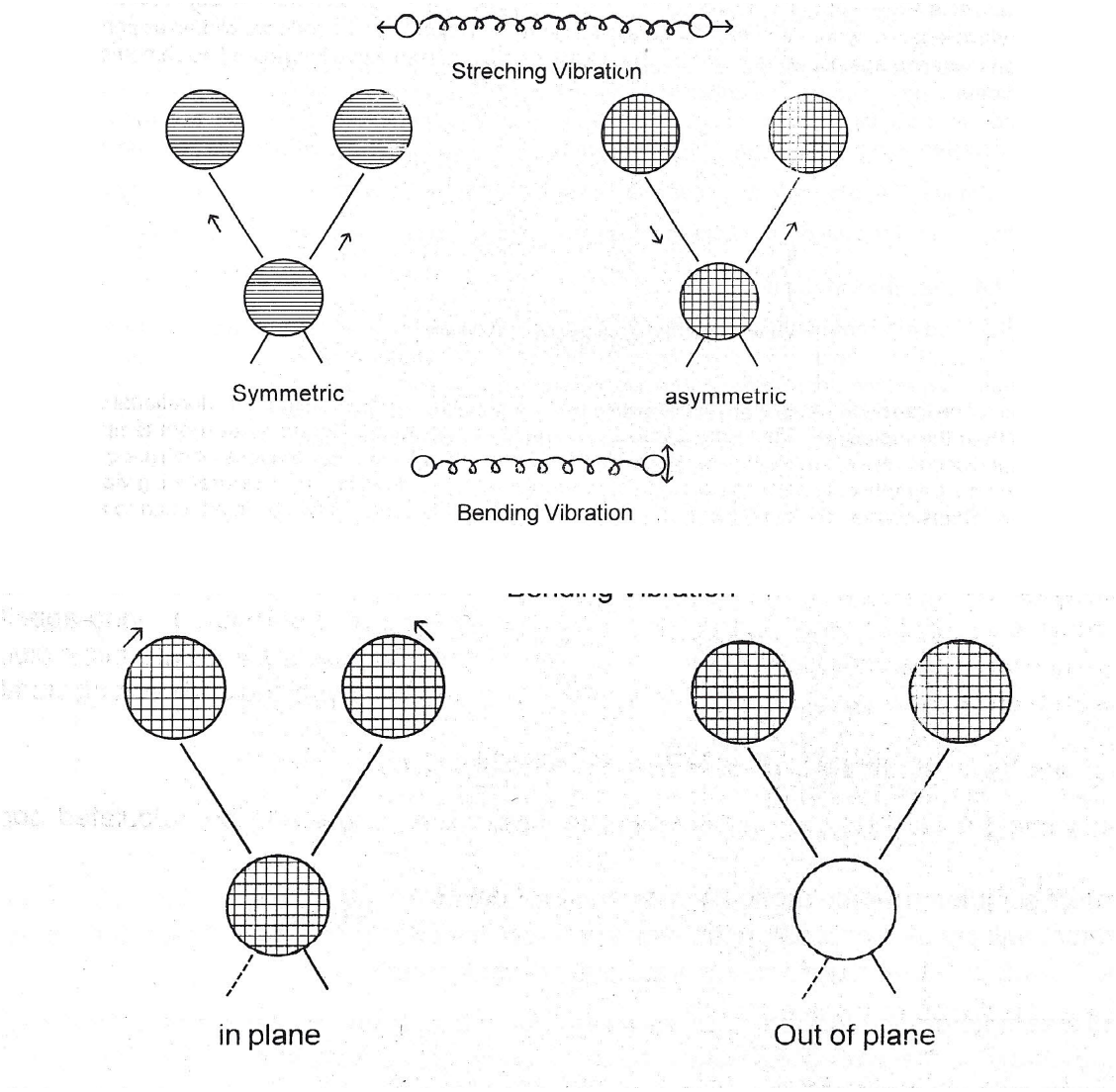


Figure 18.1: Stretching and Bending Vibrations

Some of the vibrations may have the same frequency - degenerate and their bands may overlap, some vibrations may not be intense because a particular vibration should produce fluctuating dipole for it to be IR active. Otherwise, it cannot interact with this fluctuating electric fields of the infrared light. So, for vibration to be noticed in the spectrum it should lead to a change in the dipole moment of the molecule. Otherwise, these vibrations will be IR inactive and will show less or no absorption. The absorption band intensity depends on the difference between the dipole moments of the molecule in the ground state and the vibrational excited state. Molecules with center of symmetry are IR inactive.

Ex: Carbon dioxide CO_2

O=C=O /

Overtone bands appear as weak at the integral multiplets of fundamental vibrations. A fundamental vibration at 800 cm^{-1} will give an overtone band at 1600 and 2400 $cm^{-1}$with decrease of intensities ($\frac{1}{10}$ to$\frac{1}{100}$). Vibrational coupling occurs between two bands vibrating with similar frequencies if the bands are close in the molecule. A fundamental vibration may couple with overtone of some other vibration and this coupling is called Fermi resonance. Fermi resonance takes place when the overtone and fundamental vibration have the same symmetry property and they must be predominantly associated with a group of atoms in the same part of the molecule n-butyl vinyl ether shows Fermi resonance. The overtone of fundamental vibration at 810 cm^{-1} coincides with a band at 1640 cm^{-1}. The mixing of two bands in accordance with fermi resonance gives two bands at 1640 and 1630 cm^{-1}.

18.3. INSTRUMENTATION OF INFRARED SPECTROSCOPY:

A rod of silicon carbide or Nernst filament is heated electrically in the range of 1100-1800^oCtoproduce IR radiations. The pulse beam is divided into two beams as reference beam and sample beam. When the beam passes through the sample, it becomes less intense due to absorption of certain frequencies. Now there is difference in these intensities of the two beams. Let I_o be the intensity of the reference beam and I be the intensity of the beam after interaction with the sample.

$$\text{Absorbance } A = \log\left(\frac{I_o}{I}\right)$$

$$T = 1/ I_o$$

$$A = \text{Log } (1/T)$$

The detector (bolometer) changes its resistance upon heating with the radiation. The change in the temperature will cause an unbalanced signal across this circuit and the signal will be amplified and recorded. Poly styrene is used to calibrate this spectral chart.

The solid sample can be loaded by mixing with KBr in disc form. A blank disc is prepared with pure KBr and placed in the path of reference beam. KBr is transparent to the infrared region and a complete spectrum can be scanned by mixing 2% of solid sample with it. The solid samples can also be determined as a mull or a paste in najol. Liquid samples placed as a thin film between two sodium chloride plates which are transparent to IR radiation. It is also most convenient to determine the IR spectrum in solution. Good solvents are those which have poor absorptions in IR region. Like $CHCl_3$, CCI_4, CS_2etc. are good solvents in IR. FT IR instrument contains a number of added advantages. A monochromator is not necessary in FT IR. Data undergo analog to digital conversion. Results of several scans are combined and hence a neat spectrum for small amount of sample can be obtained (**Figure 18.2** and **Figure 18.3**).

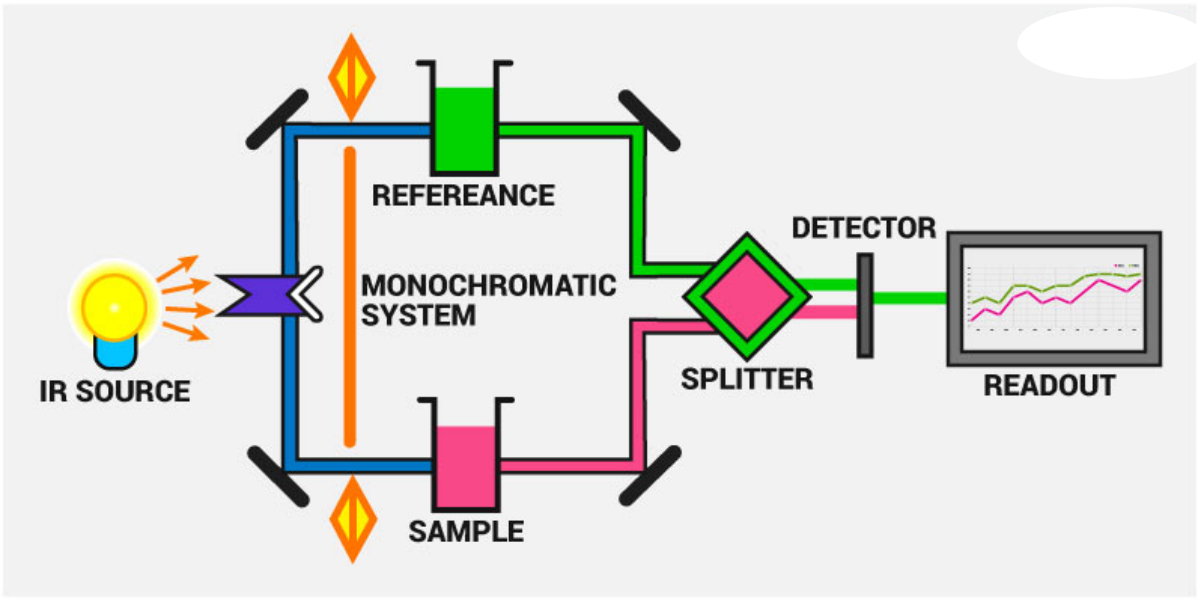


Figure 18.2: Instrumentation of IR Spectrophotometer

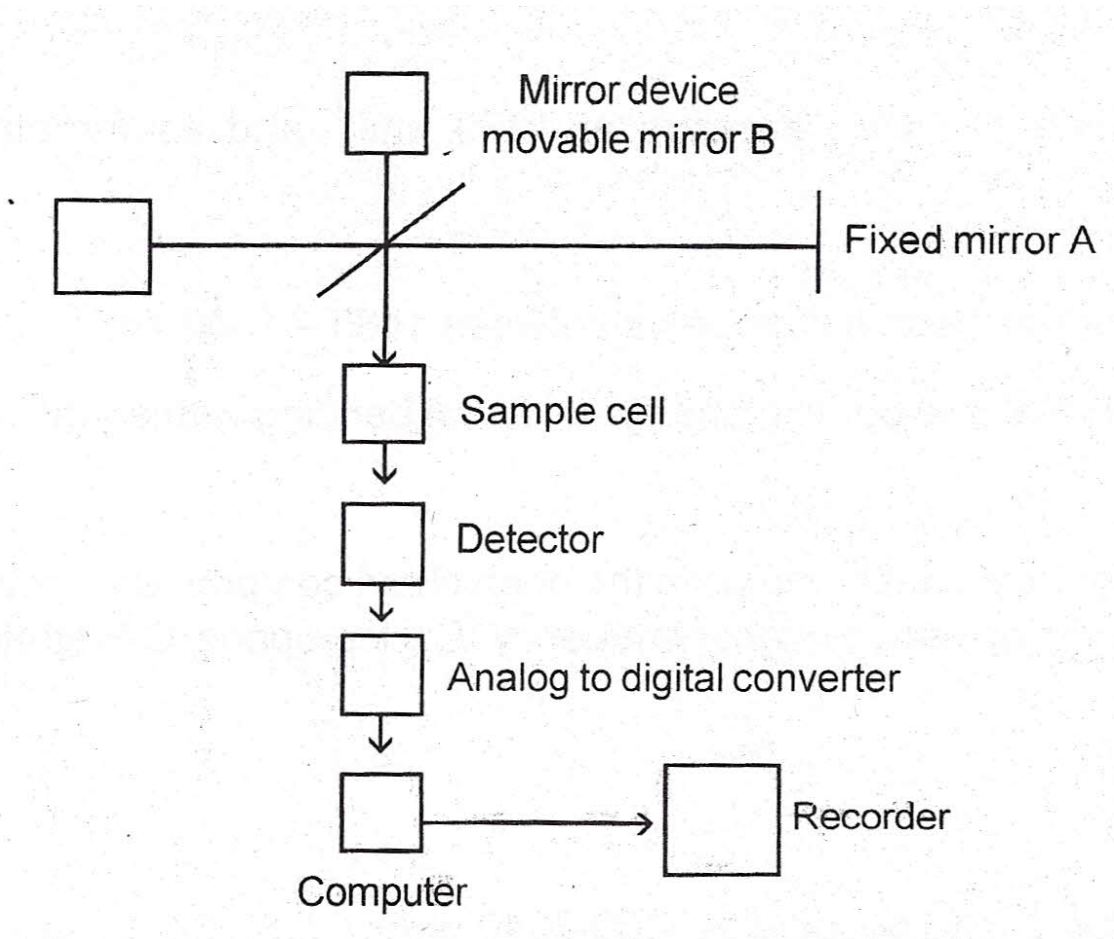


Figure 18.3: Schematic Representation of FT IR Spectrometer

The main parts of the IR spectrometer are as follows:

1) Radiation source
2) Sample cells and sampling of substances
3) Monochromators
4) Detectors
5) Recorder

1. IR radiation Sources:

IR instruments require a source of radiant energy which emits IR radiation which must be steady, intense enough for detection, and extend over the desired wavelength.

Various sources of IR radiations are as follows.

a) Nernst glower
b) Incandescent lamp
c) Mercury arc
d) Tungsten lamp
e) Glober source
f) Nichrome wire

2. Sample Cells and Sampling of Substances:

IR spectroscopy has been used for the characterization of solid, liquid, or gas samples.

a) Solid – Various techniques are used for preparing solid samples such as pressed pellet technique, solid run in solution, solid films, mull technique, etc.
b) Liquid – Samples can be held using a liquid sample cell made of alkali halides. Aqueous solvents cannot be used as they will dissolve alkali halides. Only organic solvents like chloroform can be used.
c) Gas– Sampling of gas is similar to the sampling of liquids.

3. Monochromators:

a) Various types of monochromators are prism, gratings and filters.
b) Prisms are made of Potassium bromide, Sodium chloride or Caesium iodide.
c) Filters are made up of Lithium Fluoride and Diffraction gratings are made up of alkali halides.

4. Detectors:

- Detectors are used to measure the intensity of unabsorbed infrared radiation.
- Detectors like thermocouples, Bolometers, thermisters, Golay cell, and pyro-electric detectors are used.

5. Recorders:

a) Recorders are used to record the IR spectrum.

18.4. FINGER PRINT REGION:

The IR region 500-1500 cm^{-1} is rich in many absorptions which are caused by bending vibrations and the C-C, C-O and C-N stretching vibrations. Compounds contain the same functional group show similar absorption in functional group region (1500- 4000 cm^{-1}) but their spectra differ in finger print region. Fingerprint is unique for humans. Similarly, the fingerprint region in IR spectra is unique for different compounds. No two different compounds will have the similar pattern in the finger print region of IR spectrum. If two finger print regions are similar, it means the two spectra belongs to the same compound (**Figure 18.4**).

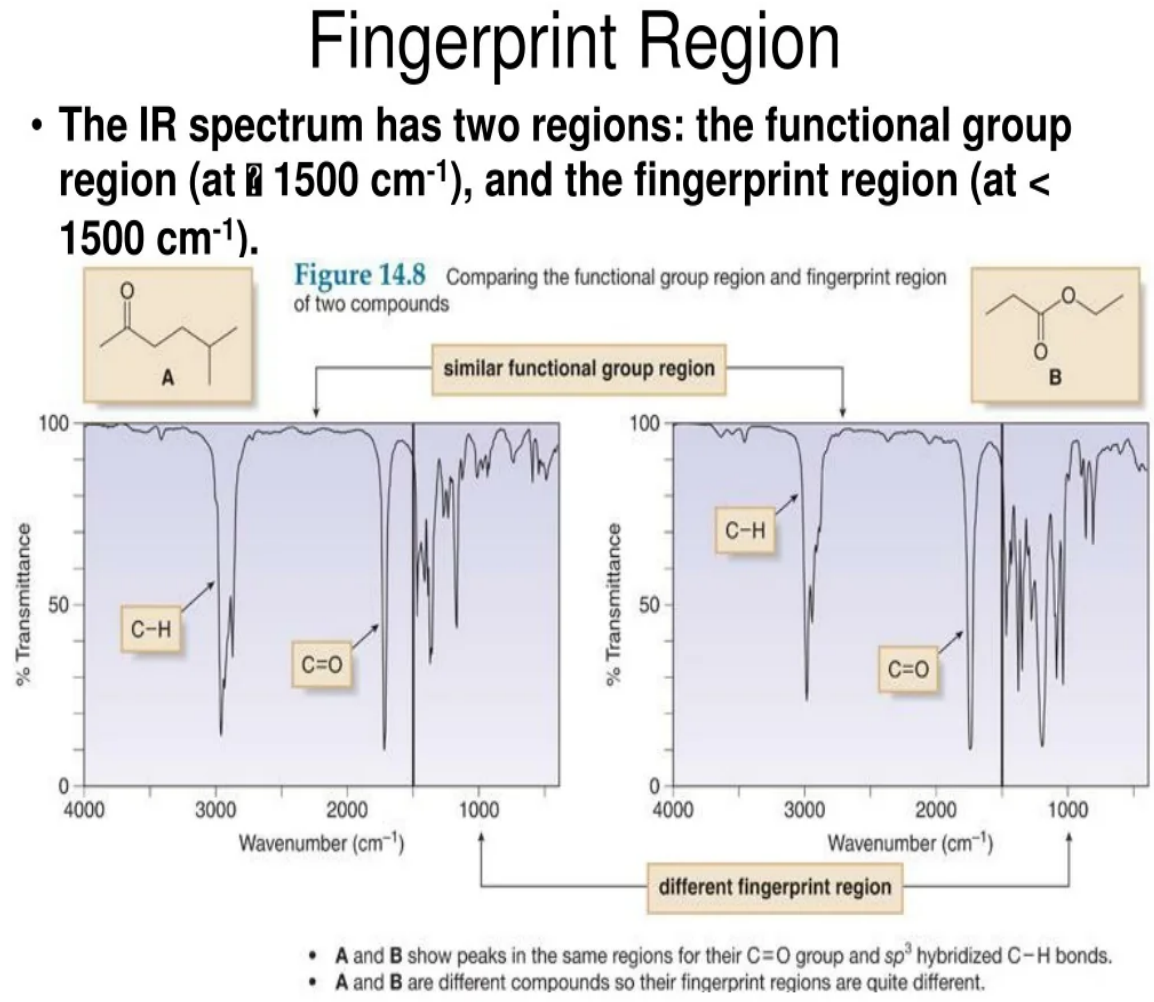


Figure 18.4: Comparing the Functional Group Region and Fingerprint Region of Two Compounds A and B.

18.5. GENERAL IR FREQUENCIES FOR DIFFERENT BONDS:

<table>
<tr><td></td><td>O-H
N-H</td><td>C-H
O=C-H</td><td>CC</td><td>C=C
C=N
C-C</td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td>Ar-H
=C-H</td><td></td><td>CN</td><td></td><td>C-O</td><td></td><td></td><td></td></tr>
<tr><td colspan="5">4000 3500 3000 2500 2000 1500
Functional group region</td><td colspan="4">1500 1000 500 cm^{-1}
Finger print region</td></tr>
</table>

18.6. APPLICATIONS OF INFRARED (IR) SPECTROSCOPY:

It has been of great significance to scientific researchers in many fields such as:

- Protein characterization
- Nanoscale semiconductor analysis and
- Space exploration.
- Analysis of gaseous, liquid or solid samples
- Identification of compounds
- Quantitative analysis
- Information regarding functional groups of molecules and constitution of molecules can be deduced from IR spectrum
- To know about interaction among molecules

18.7. LIMITATIONS AND CHALLENGES OF IR SPECTROSCOPY:

While infrared (IR) spectroscopy is an invaluable tool in various scientific fields, it is not without its limitations and challenges. Understanding these constraints is essential for researchers to maximize the technique's effectiveness and interpret results accurately. Some key limitations include:

- **Water Interference:** One of the most significant challenges in IR spectroscopy arises from water vapors, which have strong absorption features in the IR region. This can lead to significant interference when analyzing samples that contain moisture. For instance, water's broad absorption bands often obscure the spectral features of organic compounds, complicating the interpretation of results.

- **Sample Preparation Sensitivity:** Proper sample preparation is crucial for obtaining reliable spectra. Inadequate preparation, such as inconsistent sample thickness or contamination, can introduce artifacts or distort spectral data. As Jenkins et al. (2023) note,

"The intricacy of sample preparation techniques is paramount for achieving reproducible and reliable IR spectra."

- **Lack of Sensitivity for Low-Concentration Analytes:** IR spectroscopy may struggle with identifying compounds present at low concentrations. Although advanced techniques have improved sensitivity, compared to methods like mass spectrometry, IR spectroscopy can still fall short when detecting trace levels of substances. This limitation can hinder the analysis of complex matrices where low-abundance compounds are crucial.
- **Ambiguity in Spectral Interpretation:** The interpretation of IR spectra can sometimes be ambiguous, especially when overlapping peaks occur. Complex mixtures can give rise to challenging spectra that require expert knowledge for accurate identification. Furthermore, while characteristic peaks indicate the presence of specific functional groups, it can be difficult to ascertain the entire molecular structure based solely on an IR spectrum.
- **Limited Range of Identifiable Compounds:** IR spectroscopy primarily identifies bonds that are polar and lead to a change in dipole moment. As a result, homonuclear diatomic molecules (e.g., N_2, O_2) and certain symmetric compounds may be detected weakly or not at all, limiting the technique's applicability in certain situations.

18.8. SUMMARY:

- To know about the Infrared Spectroscopy.
- To study about basic Principle involved in Infrared Spectroscopy.
- To study about Instrumentation of Infrared Spectroscopy.
- To learn about molecular vibrations in Infrared Spectroscopy.
- To know about importance of Finger print Infrared Spectroscopy.
- To study about Advantages, Disadvantages and Applications of Uv-visible Spectroscopy.
- To learn about frequency ranges of various functional groups using Infrared Spectroscopy.

18.9. SELF-ASSESSMENT QUESTIONS:

1) Write the basic principle involved in IR spectroscopy.
2) Explain the instrumentation of IR spectroscopy.
3) Write the importance of Finger print region in IR spectroscopy.

4) write stretching and bending vibrations.

5) Write the basic principle and applications of IR spectroscopy.

18.10. REFERENCE BOOKS:

1) Introduction to Organic Laboratory Techniques-D.L. Pavia, G.M. Lampman, G.S. Kriz and R. G. Engel, Saunders College Pub. (NY).

2) Instrumental methods of Chemical Analysis by B.K. Sharma, Goel Publish House, Meerut.

3) Instrumental methods of Chemical Analysis by H. Kaur, Pragati Prakasan, Meerut.

4) A Hand Book of Instrumental Techniques for Analytical Chemistry- Ed-F. A. Settle, Prearson Edn.

Dr. K. Chandra Mohan

LESSON-19.1

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

19.1.0. OBJECTIVES:

After studying this lesson, you should be able to:

- To know about the NMR Spectroscopy.
- To study about basic Principle involved in NMR Spectroscopy.
- To study about Instrumentation of NMR Spectroscopy.
- To learn about chemical shift in NMR Spectroscopy.
- To know about importance of NMR in medical diagnostics NMR Spectroscopy.
- To study about Advantages, Disadvantages and Applications of NMR Spectroscopy.
- To know about chemical shift ranges in NMR Spectroscopy.

STRUCTURE:

19.1.1 Introduction

19.1.2 Instrumentation of NMR Spectroscopy

19.1.3 Chemical shift

19.1.4 Chemical Exchange

19.1.5 NMR Instrumentation

19.1.6 Applications of NMR Spectroscopy

19.1.7 Limitations of NMR spectroscopy

19.1.8 NMR in Medical Diagnostics

19.1.9 Summary

19.1.10 Self-Assessment Questions

19.1.1 INTRODUCTION:

Nuclear magnetic resonance spectrometry is basically absorption spectrometry just like UV or IR spectroscopy under appropriate conditions in a magnetic field, a sample can absorb electromagnetic radiation in the radio frequency (rf) region. A plot of frequencies of this absorption peaks versus peak intensities constitutes an NMR spectrum. All nuclei carry a charge and in some nuclei this charge' spins' on the nuclear axis, and this circulation of nuclear charge generates a magnetic dipole along the axis. The angular momentum of the spinning charge can be described in terms of nuclear spin quantum numbers(I) and they have values of 0, $\frac{1}{2}$, 1, $\frac{3}{2}$ etc ... The spin number (I) can be determined from the atomic mass and atomic number.

	Atomic Mass	Atomic Number	Examples
Half Integer	Odd	Odd or Even	${}_{1}^{1}H\ (\frac{1}{2})$, ${}_{8}^{17}O,\ (\frac{5}{2})$, ${}_{7}^{15}N\ (\frac{1}{2})$
Integer	Even	Odd	${}_{1}^{2}H\ (1)$, ${}_{7}^{14}N(1)$, ${}_{5}^{10}B(3)$
Zero	Even	Even	${}_{6}^{12}C(0)$, ${}_{8}^{16}O(0)$, ${}_{16}^{32}S(0)$

Several nuclei (${}_{1}^{1}H$, ${}_{1}^{3}H$, ${}_{6}^{13}C$, ${}_{7}^{15}N$, ${}_{9}^{19}F$, ${}_{9}^{31}P$)have spin number I of $\frac{1}{2}$and a uniform spherical charge distribution. Nuclei with spin number I of 1 or higher have non-spherical charge distribution. Such a magnetically active nucleus is placed in an external magnetic field, the nuclei, tiny bar magnets, will take up definite orientation which is quantized. The number of possible orientations will be given the equation N = 2I + 1, where I is the spin number of nucleus under consideration. Each orientation s associated with definite energy and the energy of each state can be defined. The energy difference between adjacent levels can be given by this expression,

$\Delta E = h \nu$

h =Planks constant

ν= Frequency of electromagnetic radiation

It is found that $\omega = (\gamma/2\pi)\ H_0$

ω = angular precessional velocity

H_o- Applied field in gauss

γ- Gyromagnetic ratio = $\frac{2\pi\mu}{hI}$

μ = Magnetic moment of the nuclei

I = Spin number of the spinning magnetic

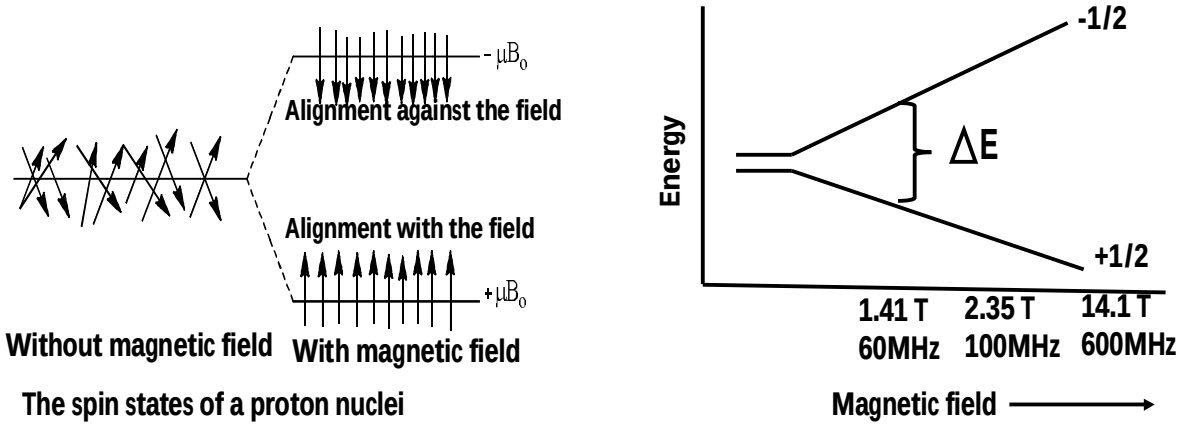
.. '. .:. According to the fundamental NMR equation which correlates electromagnetic frequencies with the magnetic field we say

$\nu\ H_o = 2\pi\nu$

ν = Frequency of electromagnetic radiation.

The value of the frequency ν= inserted is called processional frequency.

For a nucleus with I=1/2 the number of possible orientations in a magnetic field will be (2I+1) 2 (for^{1}H, ^{l3}C, ^{19}F) and taking up one of the two orientations with respect to the external field.



When a proton is pressing this aligned orientation, it can pass into the opposed orientation by absorbing energy. From the high energy opposed orientation it comes back to the low energy aligned orientation by losing energy.

The transition from one energy state to the other 'is called flipping of the proton. The transition between two energy states can be brought about by the absorption of quantum of electromagnetic radiation in the radio wave region with energy hν. The energy required to bring about the transition depends upon the strength of this external field. Stronger the field greater will be the tendency of the nuclear magnet to remain lined up with it and higher frequency of radiation needed to flip the proton to the higher energy state.

We know that $$\nu=\frac{\gamma H_o}{2\pi}$$

ν= Frequency in cycles per see or Hz

H_o = Strength of the magnetic field in gauss

γ = Nuclear constant or Gyromagnetic ratio and is equal to 26750 for ^{1}H 6728 for ^{13}C.

At 14092 gauss (1.4T) this energy required to cause flipping corresponds to electromagnetic radiation of frequency 60 million cycles per second or 60 MHz. When we irradiate these precession nuclei with a beam of electromagnetic radiation of desired frequency, then the low energy nuclei will absorb it and move to higher energy state. The precession proton will absorb energy from the radiofrequency region only if the precession frequency is the same as the frequency of the radio frequency beam, then the nucleus and radio frequency beam are in resonance. Hence it is called Nuclear Magnetic Resonance.

Relaxation Process:

When the population in the two energy states becomes equal, equilibriums established and thereafter no further absorption of energy by the nuclei. However, there is a process known as relaxation which retains the small excess population in the lower energy state throughout the irradiation process.

Relaxation process involves non-radiative transitions by which a nucleus in an upper transition state returns to lower spin state. There are two kinds of such relaxation process.

i) Spin - Spin relaxation

ii) Spin -lattice relaxation

In the former 'process the nuclei energy is exchanged between lower energy state and higher energy state in the surrounding nuclei. In this latter process the nuclei lose their energy to the surroundings, to the lattices.

19.1.2 INSTRUMENTATION OF NMR SPECTROSCOPY:

In NMR spectrophotometer a magnet, a radio frequency, a detector, and an Amplifier are important parts (**Figure 19.1**). The sample in a glass tube is placed between the pole faces of a magnet. A R_f source is made to fall on this sample. A signal is detected if the nuclei resonate with the source, i.e. energy 'required to flip the proton is the same as that of the source. The spectrum can be obtained either by CW scan or pulse FT. Generally, protons in a molecule being in different electronic environments cannot resonate at the same frequency. For practical purposes the radio frequency source is held constantly and field strength is varied. Peaks are measured by an electronic integrator that traces the height proportional to the peak area. Peak positions are measured in frequency units from a reference peak.

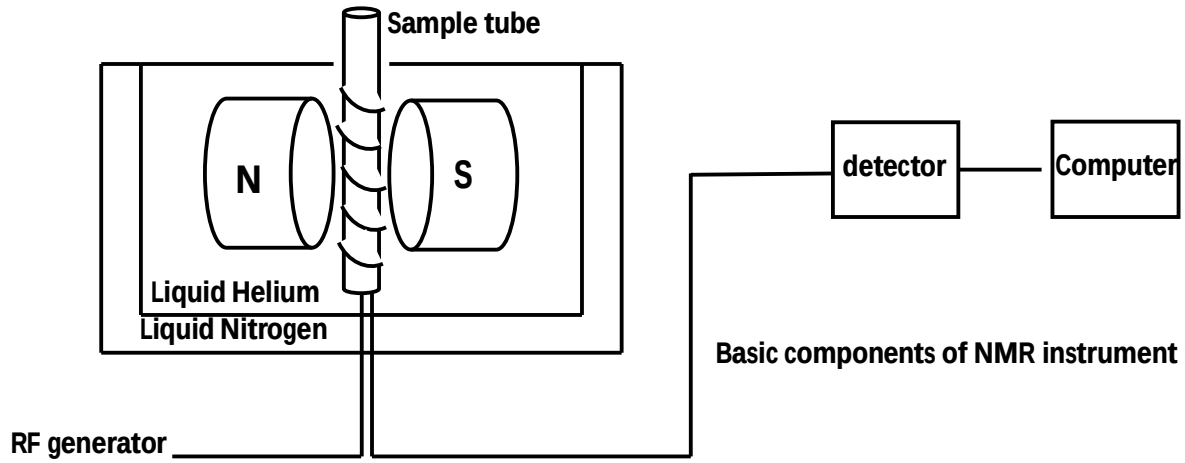


Figure 19.1: Basic Components of NMR Instrument

19.1.3 CHEMICAL SHIFT:

The nucleus is shielded to a small extent by its electron cloud and whose density varies with this environment. This variation gives rise to different absorption positions usually within the range of about 750 Hz in a magnetic field corresponding to 60 MHz. The

electrons under the influence of a magnetic field will circulate, and will generate their own magnetic field opposing the applied field. This is called local diamagnetic shielding effect. The degree of Shielding depends on the density of the circulating electrons and on the inductive effect of other groups attached to these carbon atoms. The difference in the absorption position of a particular proton from the absorption position of a reference proton is called as chemical shift of that particular proton. Generally, Tetramethylsilane is used as reference compound. The δ scale generally ranges from 0 to 14 and sometimes even negative for antiaromatic protons (**Table 19.1**).

$$\text{Chemical shift, } \delta = \frac{\text{Frequency of the compound (Hz)} - \text{frequency of the reference (Hz)}}{\text{Frequency of the instrument (MHz)}} \qquad \delta = \frac{\nu_c - \nu_{TMS}}{\nu_o} \text{ ppm}$$

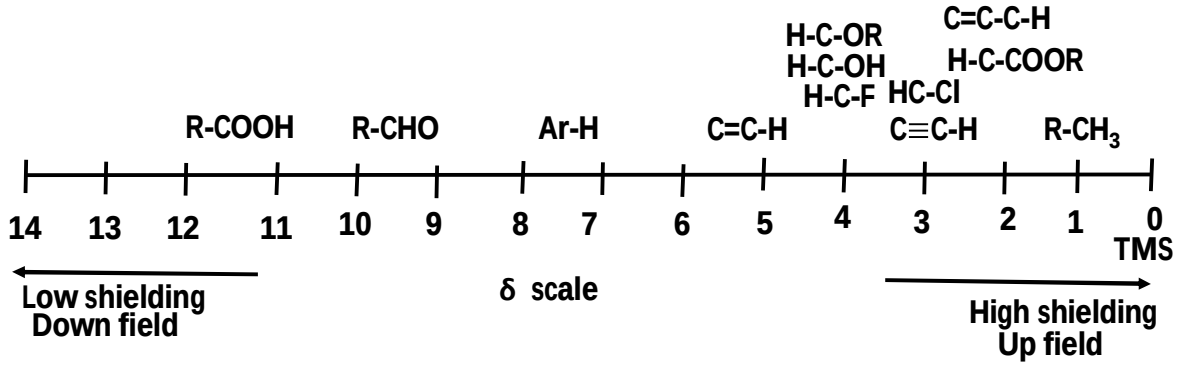
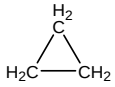


Table 19.1: Chemical Shift for Various Types of Protons

Types of Protons	Formula	Chemical shift in PPM	
		δ	τ
Cyclopropane		0.2	9.8
Primary	R-CH_3	0.9	9.1
Vinylic	C=C-H	4.6-5.8	5.4-4.2
Acetylenic	C≡C-H	2-3.5	8-6.5
Aromatic	Ar-H	6-9.0	4-1.0
Fluorides	H-C-F	4-4.5	6-5.5
Chlorides	HC-Cl	3-4	7-6

Alcohols	H-C-OH	3.4-4	6.6-6
Ethers	H-C-OR	3.3-4	6.7-6
Esters	H-C-COOR	2-2.2	8-7.8
Acids	H-C-COOH	2-2.5	8-7.5
Aldehydic	R-CHO	9-01	1-0
Hydroxyl	R-OH	1-5.5	9-4.5
Phenolic	Ar-OH	4-12	6 to 12
Enolic	C=C-OH	15-17	-5 to -7
Carboxylic	RCOOH	10.5-12	-0.5 to -2

19.1.4 CHEMICAL EXCHANGE:

In a molecule, if a proton shuttles between two magnetic environments at a rate which is much faster in comparison with the NMR transition time then the resonance observed for that proton will be simply that of the average effective field in the two environments thus only are resonance will be observed, although the proton will shuttle in the different magnetic environments. We know that the-OH proton in water has different chemical shift as compared to that of the -OH proton in acetic acid in water at an average position according to the following formula.

$$N_a \delta_a + N_b \delta_b$$

Where

N_a= Mole fraction of the proton a

N_b = Mole fraction of the proton b

δ_a= Chemical shift of unexchanged proton a

δ_b= Chemical shift of unexchanged proton b

This clearly shows that the rate at which the -OH proton exchanges between water and acetic acid is much faster than the nuclear transition time.

The phenomenon of chemical exchange can be explained by considering the NMR spectrum of anhydrous ethanol and the spectrum of ethanol containing small quantities of water.

In the case of pure anhyrides ethanol CH_3- CH_2- OH three signals are observed.

1) A triplet for CH_3Protons at 8.827due to coupling with CH_2protons.
2) A multiplet consisting of light lines for - CH_2 - protons at 6.38, the - CH_2- protons are under the influence of two kinds of proton in different chemical environments. Thus, the multiplet consist (n+1) (n^1+1) = (3+1) (1+1) = 8 lines.
3) A triplet for -OH proton at 4.72. The OH proton appears as a triplet because of coupling to - CH_2-Protons.

Now if we scan the spectrum of ethyl alcohol containing water, the OH signals appear as singlet and its coupling with adjacent – CH_2- does not take place.

The alcohol containing some amount of water, a singlet is observed for the hydroxyl proton. The position of the singlet peak for the OH proton is slightly shifted depending upon the water content in alcohol and hence, it helps in the quantitative analysis of ethanol- water, acetic acid - water mixtures.

The spin decoupling can usually be observed at 1) high temperature and 2) by using highly polar solvents for the polar samples.

The spectrum of methanol (CH_3OH) at very low temperature (- 40°) shows a quartet for hydroxyl proton and a doublet for methyl protons. This shows that the chemical exchange is very slow at –40°C as compared to the NMR transition time. But if the temperature is raised to + 31 °C multiplets CH_3 and -OH protons collapse to the sharp singlets. Thus, shows that the rate of chemical exchange becomes faster than the NMR transition time.

19.1.5 NMR INSTRUMENTATION:

An example of an NMR spectrometer is given in **Figure 1**. NMR spectroscopy works by varying the machine's emitted frequency over a small range while the sample is inside a constant magnetic field. Most of the magnets used in NMR machines to create the magnetic field range from 6 to 24 T. The sample is placed within the magnet and surrounded by superconducting coils, and is then subjected to a frequency from the radio wave source. A detector then interprets the results and sends it to the main console (**Figure 19.2**).

Sample Holder:

In NMR spectroscopy, a typical NMR sample cell is 8.5 cm long and 0.3 cm in diameter. The sample holder should be chemically inert, long-lasting, and Rf radiation-transparent.

Glass tubes are often strong, practical, and inexpensive. For high-resolution spectra, the sample must be in a liquid or solution state.

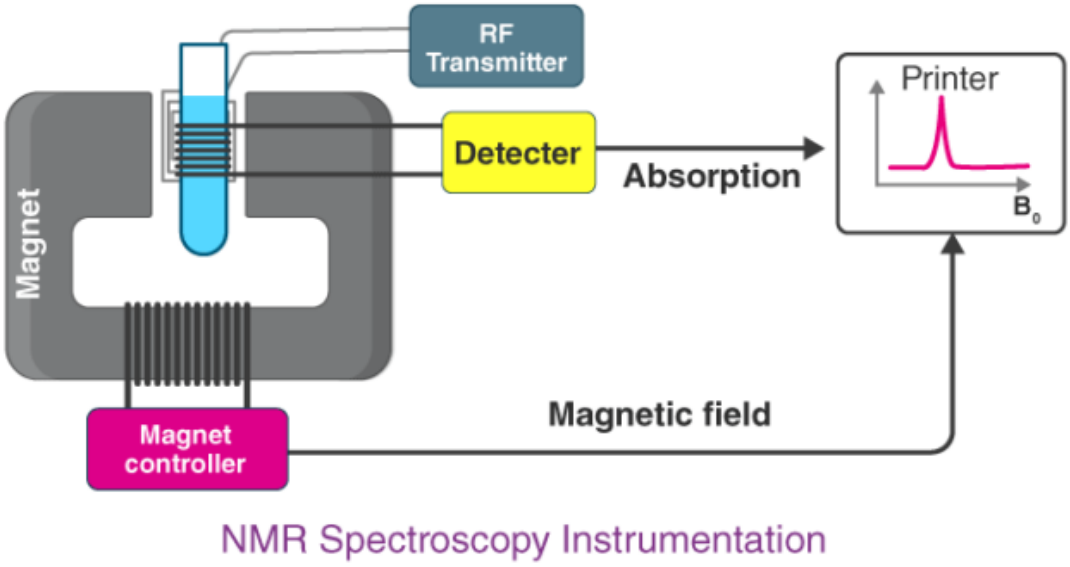


Figure 19.2: Instrumentation of NMR Spectroscopy

Magnet:

The magnet should produce a uniform magnetic field. The magnetic field strength is large because chemical changes are related to field strength. Two criteria are critical in magnet design:

i) field homogeneity or uniformity and field strength consistency

ii) maximum achievable field strength.

Permanent and conventional electromagnets are commonly utilized in spectrometers operating at frequencies ranging from 100 to 230 MHz.

Magnetic Coil:

A pair of coils (Helmholtz coils) positioned parallel to the magnet face can be used to change the applied field over a short range. The field intensity varies automatically and linearly over time, and this variation is synchronized with a linear device or a chart recorder.

Sweep Generator:

It alters the strength of a previously applied magnetic field. The precession frequency of a nucleus must be equal to that of the applied field or radiofrequency radiation in order for the nucleus to resonate. This can be accomplished by:

a) Frequency sweep method: The frequency sweep approach is used to resonate the nucleus. The frequency of the radiofrequency radiation is altered so that it equals the resonance frequency or the precession frequency.

b) Field Sweep Method: In this method, the frequency of the radiofrequency radiation is held constant while the precession frequency is varied by varying the applied magnetic field. It is preferable to radiofrequency radiation because it is easy to alter the H_O.

Radiofrequency Generator:

A radio frequency generator is used to generate radio frequency. Normally, a fixed oscillator with a capacity of exactly 60 MHz is employed.

To maximize radiofrequency radiation interaction with the sample, the oscillator coil is looped around the sample container. The coil is wound perpendicular to the applied magnetic field.

Detector and Recorder:

The coil was used to direct the radio frequency signal generated by the resonating nuclei. The electrical signal generated in the coil must be amplified before it can be recorded. The detector assists in determining the unabsorbed radio frequencies. The NMR signals received by the RF detector are recorded by the recorder.

19.1.6 APPLICATIONS OF NMR SPECTROSCOPY:

- Organic, organometallic, and biological compounds are identified and structurally elucidated by using NMR spectroscopy.
- In biophysics and molecular biology, NMR spectroscopy is used to investigate the structure, dynamics, and molecular interactions of biomolecules such as peptides, proteins, nucleic acids, carbohydrates, and others.
- NMR spectroscopy is also employed in biological fluid analysis to acquire metabolic profiles related to disorders (metabolomics), as well as in NMR imaging techniques for medical diagnosis.
- NMR fingerprint analysis is used to verify the quality or authenticity of food, wine, and cannabis samples.
- In the NMR spectrum, each functional group has a distinct signal. The functional group in the compound can be identified by observing the chemical shift of the substance.
- It is feasible to place hydrogen at a suitable position in the formula and so determine the structure of a given molecule by observing the chemical shift values and splitting of the signal of protons under different conditions.
- NMR spectroscopy may be used to assess molecule conformation in solutions as well as analyze physical features at the molecular level such as conformational exchange, phase shifts, solubility, and diffusion once the basic structure is understood.
- NMR spectroscopy can quantify mixtures containing known chemicals. For unknown substances, NMR can be utilized to compare against spectral libraries or to derive the basic structure directly.
- NMR spectroscopy is used in pharmaceutics to examine the structure, dynamics, and molecular interactions of pharmaceuticals for drug discovery, quality control, and purity determination.
- It is used in petrochemistry for rock material analysis to determine the appropriateness of an oil reservoir for extraction, solid-state NMR composition analysis of petroleum derivatives, and product quality control.

- NMR spectroscopy is also used for the characterization of novel materials.
- NMR spectroscopy is used to investigate dynamic features of molecules such as conformational isomerism, molecular asymmetry, hydrogen bonding, and so on.
- It is used to determine the optical purity.
- NMR spectroscopy is used to investigate drug-receptor interactions.

19.1.7 LIMITATIONS OF NMR SPECTROSCOPY:

- NMR spectroscopy is a crucial method in organic chemistry. However, there are some limitations of this method that should be considered while employing it.
- NMR spectroscopy is a versatile approach; however, it sometimes fails due to its low sensitivity. This is a significant drawback when investigating metabolomics or other complex reactions.
- Drift in the magnetic field has a significant impact on NMR spectroscopy, resulting in deformed lines and spectral leakage. This can make interpreting results exceedingly challenging.
- The sample must be NMR active.

19.1.8 NMR IN MEDICAL DIAGNOSTICS:

The NMR imagining method is generally acknowledged to be superior to the existing imagining techniques such as CAT scanners and X-ray machines because

i) The irradiation frequencies are very low in energy and currently believed to be harmless to human tissues and organs.

ii) The rapidity with which individual images can be accumulated and stored makes this technique an important source of information on physiological functions of the body organs such as kidneys, heart, brain etc.

iii) NMR metabolomics is used in the search for biomarkers of infectious diseases like tuberculosis, malaria and pneumonia, neurological disorders and Parkinson's disease.

iv) NMR metabolomics has been used in the identification of biomarker for cardiovascular diseases and risk stratification. It has also been used in cancer diagnosis and therapy.

v) NMR metabolomics has been used to investigate processes like transformation, progression, proliferation and metastasis in cancer cell lines.

vi) NMR metabolomics include gastro-intestinal disorders, endocrine and nutritional disorders, disorders of the nervous system and respiratory system disorders.

19.1.9. SUMMARY:

- To know about the NMR Spectroscopy.
- To study about basic Principle involved in NMR Spectroscopy.
- To study about Instrumentation of NMR Spectroscopy.

- To learn about chemical shift in NMR Spectroscopy.
- To know about importance of NMR in medical diagnostics NMR Spectroscopy.
- To study about Advantages, Disadvantages and Applications of NMR Spectroscopy.
- To know about chemical shift ranges in NMR Spectroscopy.

19.1.10 SELF-ASSESSMENT QUESTIONS:

1) Write the basic principle involved in NMR spectroscopy.
2) Write the instrumentation of NMR spectroscopy.
3) Discuss the chemical shift in NMR spectroscopy.
4) Write the importance of NMR in medical diagnostics.
5) Write the applications and limitations of NMR spectroscopy.

19.1.11. REFERENCE BOOKS:

1) Introduction to Organic Laboratory Techniques-D.L. Pavia, G.M. Lampman, G.S. Kriz and R. G. Engel, Saunders College Pub. (NY).
2) Instrumental methods of Chemical Analysis by B.K. Sharma, Goel Publish House, Meerut.
3) Instrumental methods of Chemical Analysis by H. Kaur, Pragati Prakasan, Meerut.
4) A Hand Book of Instrumental Techniques for Analytical Chemistry- Ed-F.A. Settle, Prearson Edn.
5) Principles of Instrumental Analysis by D.A. Skoog, F.J. Holler and T.A. Nieman, Harcourt College Pub.

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LESSON-19.2

ELECTRON SPIN RESONANCE SPECTROSCOPY

(OR)

ELECTRON PARAMAGNETIC RESONANCE SPECTROSCOPY

19.2.0. OBJECTIVES:

After studying this lesson, you should be able to:

- To know about the ESR Spectroscopy.
- To study about basic Principle and working principle involved in ESR Spectroscopy.
- To study about Instrumentation of ESR Spectroscopy.
- To study about Advantages, Disadvantages and Applications of ESR Spectroscopy.

STRUCTURE:

19.2.1 Introduction

19.2.2 Principle of ESR Spectroscopy

19.2.3 Working of ESR Spectroscopy

19.2.4 Instrumentation of Electron Spin Resonance (ESR)

19.2.5 Applications of ESR Spectroscopy

19.2.6 Advantages of ESR Spectroscopy

19.2.7 Limitations of ESR spectroscopy

19.2.8 Summary

19.2.9 Self-Assessment Questions

19.2.10 Reference Books

19.2.1 INTRODUCTION:

ESR spectroscopy, also known as Electron Paramagnetic Resonance (EPR) spectroscopy, is a technique used to detect transitions induced by electromagnetic radiation between different energy levels of electron spins in the presence of a static magnetic field. It is a non-destructive method widely utilized in the study of transition metal complexes and crystal geometries.

Electron spin resonance (ESR), also called electron paramagnetic resonance (EPR), is a spectroscopic technique confined to the study of species having one or more unpaired electrons.

Among the large number of systems having one or more unpaired electrons, i.e. paramagnetic system, the most important ones are free radicals, transition metal ions, ions and molecules having odd number of electrons.

19.2.2 PRINCIPLE OF ESR SPECTROSCOPY:

ESR spectroscopy is a powerful technique used to study the properties of unpaired electrons in paramagnetic species. It provides information about the electronic structure, chemical environment, and dynamics of free radicals, transition metal ions and other paramagnetic species. ESR spectroscopy is based on the absorption of microwave radiation by paramagnetic substances containing unpaired electrons when subjected to a strong magnetic field.

Paramagnetic species possess unpaired electrons, which have a spin property. When an external magnetic field is applied, the energy levels associated with different spin orientations of the unpaired electrons split, resulting in distinct energy transitions. ESR measures the absorption of energy by the sample as the applied magnetic field is varied, allowing the determination of the g-value (a measure of the electronic environment) and the line width of the signal (**Figure 19.3**).

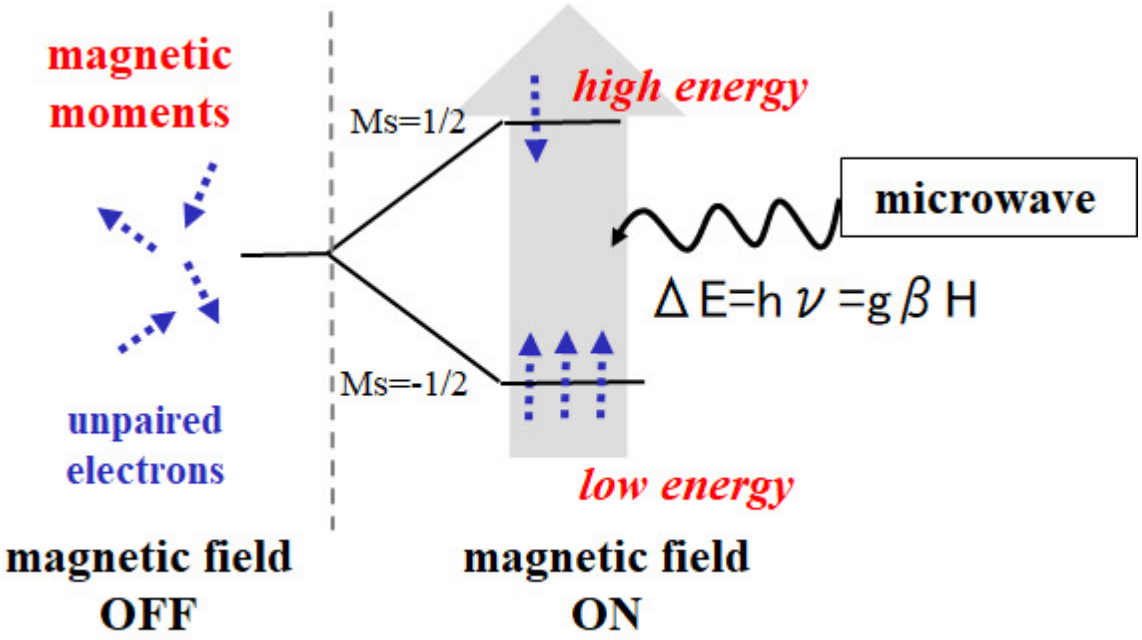

Figure 19.3: Energy Levels of Electron Spin in Presence and Absent of Magnetic Field

The key points on which the method is based are as follows:

- **Electron Behaviour**: ESR spectroscopy allows the study and measurement of the absorption of microwave energy by unpaired electrons in a magnetic field. It provides information about the behaviour of electrons in the sample under investigation.
- **Energy Level Splitting:** When a substance with unpaired electrons is placed in a magnetic field, the electronic energy levels of the atoms or molecules split into different levels. This splitting is known as **magnetic resonance absorption**.
- **Microwave Radiation**: An ESR instrument utilises a static magnetic field and microwaves to observe the behaviour of unpaired electrons in the material being studied. The microwave frequency used in ESR falls within the range of **10^4 to 10^6 MHz**.

- **Resonance Absorption**: In ESR, the static magnetic field causes a difference in energy between the electron spins with **ms = +1/2** and **ms = -1/2.** This energy difference corresponds to the resonance absorption of applied microwave energy.

19.2.3 WORKING OF ESR SPECTROSCOPY:

The working principle of ESR (Electron Spin Resonance) spectroscopy involves the interaction of paramagnetic species with a static magnetic field and microwave radiation. Here is a step-by-step explanation of the working principle:

- **Sample Preparation**: The sample under investigation is prepared, which may contain paramagnetic species such as free radicals or transition metal complexes. The sample is typically in the form of a powder or solution.
- **Magnetic Field Application:** A static magnetic field is generated using powerful magnets. The sample is placed within this magnetic field, which causes the energy levels associated with the electron spins of the paramagnetic species to split.
- **Microwave Irradiation**: Microwaves with a specific frequency range, typically in the range of 10^4 to 10^6 MHz, are applied to the sample. These microwaves carry energy that can induce transitions between the split energy levels of the paramagnetic species.
- **Resonance Condition:** The strength of the magnetic field is varied while keeping the microwave frequency constant. At a specific magnetic field strength, the energy difference between the split energy levels matches the energy carried by microwave radiation. This condition is called resonance.
- **Absorption of Microwave Energy**: When the resonance condition is met, the paramagnetic species absorbs energy from the microwaves. This absorption leads to a decrease in the microwave power passing through the sample. The amount of absorbed energy depends on factors such as the number of unpaired electrons and their environment.
- **Signal Detection:** The decrease in microwave power, resulting from the absorption of energy by the paramagnetic species, is detected and recorded by the ESR instrument.
- **Data Analysis:** The recorded ESR spectrum, which represents the absorption of microwave energy at different magnetic field strengths, is analysed. The spectrum provides valuable information, such as the **g**-value (related to the electronic environment) and the linewidth (reflecting electron mobility and interactions) of the paramagnetic species. These parameters offer insights into the electronic structure, coordination environment, and dynamics of the studied sample.

19.2.4 INSTRUMENTATION OF ELECTRON SPIN RESONANCE (ESR):

a) Klystrons:

- Klystron tube acts as the source of radiation.
- It is stabilized against temperature fluctuation by immersion in an oil bath or by forced air cooling.
- The frequency of the monochromatic radiation is determined by the voltage applied to klystron.

- It is kept a fixed frequency by an automatic control circuit and provides a power output of about 300 milli watts (**Figure 19.4**).

b) Wave Guide or Wave meter

- The wave meter is put in between the oscillator and attenuator.
- To know the frequency of microwaves produced by klystron oscillator.
- The wave meter is usually calibrated in frequency unit (megahertz) instead of wavelength.
- Wave guide is a hollow, rectangular brass tube. It is used to convey the wave radiation to the sample and crystal.

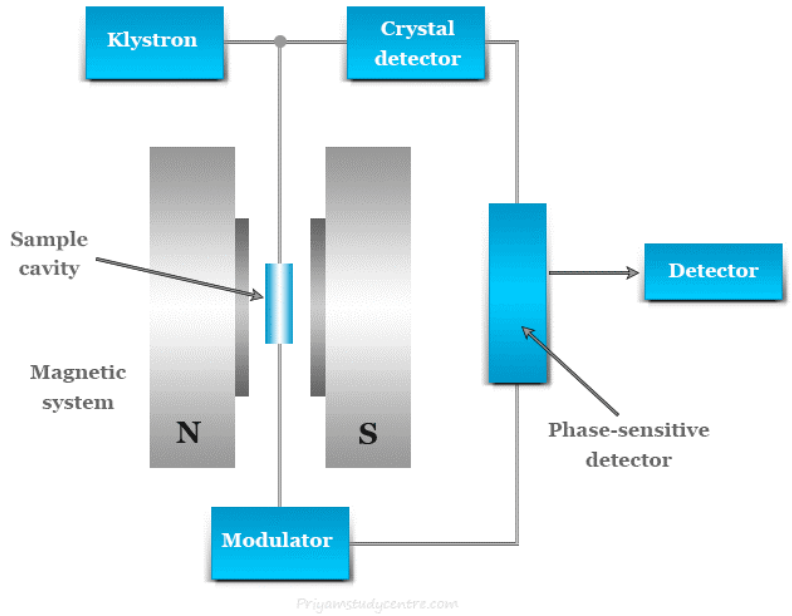

Figure 19.4: ESR Instrumentation

c) Attenuators:

- The power propagated down the wave guide may be continuously decreased by inserting a piece of resistive material into the wave guide. This piece is called variable attenuator.
- It is used in varying the power of the sample from the full power of klystron to one attenuated by a force 100 or more.

d) Isolators:

- It's device which minimizes vibrations in the frequency of microwaves produced by klystron oscillator.
- Isolators are used to prevent the reflection of microwave power back into the radiation source.

- It is a strip of ferrite material which allows micro waves in one direction only.
- It also stabilizes the frequency of the klystron.

e) Sample Cavities:

- The heart of the ESR spectrometer is the resonant cavity containing the sample.
- Rectangular TE120 cavity and cylindrical TE011 cavity have widely been used.
- In most of the ESR spectrometers, dual sample cavities are generally used This is done for simultaneous observation of a sample and a reference material.
- Since magnetic field interacts with the sample to cause spin resonance the sample is placed where the intensity of magnetic field is greatest.

f) Couplers and Matching Screws:

- The various components of the micro wave assembly to be coupled together by making use of irises or slots of various sizes.

g) Crystal Detectors:

- Silicon crystal detectors, which converts the radiation in D.C has widely been used as a detector of microwave radiation.

h) Magnet System:

- The resonant cavity is placed between the poles pieces of an electromagnet.
- The field should be stable and uniform over the sample volume.
- The stability of field is achieved by energizing the magnet with a highly regulated power supply.
- The ESR spectrum is recorded by slowly varying the magnetic field through the resonance condense by sweeping the current supplied to the magnet by the power supply.

i) Modulation Coil:

- The modulation of the signal at a frequency consistent with good signal noise ratio in the crystal detector is accomplished by a small alternating variation of the magnetic field.
- The variation is produced by supplying an A.C. signal to modulation coil oriented with respect the sample in the same direction as the magnetic field.
- If the modulation is of low frequency (400 cycles/sec or less), the coils can be mounted outside the cavity and even on the magnet pole pieces.
- For higher modulation frequencies, modulation coils must be mounted inside the resonant cavity or cavities constructed of a non-metallic material e.g., Quartz with a tin silvered plating.

j) Display Devices:

- In order to observe the signal a system is connected different devices can be used.

19.2.5 APPLICATIONS OF ESR SPECTROSCOPY:

ESR spectroscopy finds wide-ranging applications in the study of free radicals and structural determination. Here is a detailed explanation of both applications:

- **Study of Free Radicals:**
    - ESR spectroscopy allows for the investigation of free radicals, even at low concentrations.
    - It enables the identification of the structures of both organic and inorganic free radicals.
    - Molecules in the triplet state can be studied using ESR spectroscopy.
    - ESR spin-labelling provides valuable information about the polarity of the surrounding environment.
    - ESR spectroscopy is used for the identification of irradiated food, as it can detect different types of free radicals formed during the irradiation process.
    - It is also effective in detecting paramagnetic ions and free radicals in various materials.

Structural Determination:

- In certain cases, ESR spectroscopy provides insights into the shape and structural characteristics of radicals.

19.2.6 ADVANTAGES OF ESR SPECTROSCOPY:

- **Study of Paramagnetic Species:** ESR spectroscopy is specifically designed to investigate free radicals, allowing the characterisation of free radicals, transition metal complexes, and other reactive intermediates.
- **Sensitivity**: ESR spectroscopy is highly sensitive and can detect a small number of paramagnetic species, making it suitable for studying low-concentration samples.
- **Non-Destructive Technique**: ESR spectroscopy is a non-destructive technique that does not require extensive sample preparation, enabling the examination of samples without altering their properties.
- **Information-Rich**: ESR provides valuable information about the electronic structure, coordination environment, and dynamics of paramagnetic species, facilitating the understanding of their chemical and physical properties.

19.2.7 LIMITATIONS OF ESR SPECTROSCOPY:

- Limited to species with unpaired electrons
- Ineffective for diamagnetic compounds
- Overlapping signals can complicate interpretation

- Often requires low temperatures for stable measurements
- Sample matrix may distort spectral features
- Quantitative analysis can be challenging due to background noise
- Equipment is complex and expensive
- Short radical lifetimes can reduce detection sensitivity

19.2.8 SUMMARY:

- To know about the ESR Spectroscopy.
- To study about basic Principle and working principle involved in ESR Spectroscopy.
- To study about Instrumentation of ESR Spectroscopy.
- To study about Advantages, Disadvantages and Applications of ESR Spectroscopy.

19.2.9 SELF-ASSESSMENT QUESTIONS:

1) Write the basic principle involved in ESR spectroscopy.
2) Discuss the instrumentation involved in ESR spectroscopy.
3) Write the principle, Advantages, Disadvantages and Applications ESR spectroscopy.

19.2.10. REFERENCE BOOKS:

1) Introduction to Organic Laboratory Techniques-D.L. Pavia, G.M. Lampman, G.S. Kriz and R. G. Engel, Saunders College Pub. (NY).
2) Instrumental methods of Chemical Analysis by B.K. Sharma, Goel Publish House, Meerut.
3) Instrumental methods of Chemical Analysis by H. Kaur, Pragati Prakasan, Meerut.
4) A Hand Book of Instrumental Techniques for Analytical Chemistry- Ed-F.A. Settle, Prearson Edn.
5) Principles of Instrumental Analysis by D.A. Skoog, F.J. Holler and T.A. Nieman, Harcourt College Pub.

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LESSON-20.1

MICROSCOPIES STUDIES - TEM AND SEM

TRANSMISSION EMISSION MICROSCOPE (TEM)

20.1.0. OBJECTIVES:

After studying this lesson, you should be able to:

- To know about the Microscopic studies.
- To study about basic Principle involved in TEM.
- To study about Instrumentation of TEM.
- To study about Advantages, Disadvantages and Applications of TEM.

STRUCTURE:

Transmission Emission Microscope (TEM)

20.1.1 Introduction

20.1.2 Instrumentation of TEM

20.1.3 Advantages of TEM

20.1.4 Disadvantages of TEM

20.1.5 Applications of Transmission Electron Microscope (TEM)

20.1.6 Limitations of Transmission Electron Microscope (TEM)

20.1.7 Summary

20.1.8 Self-Assessment Questions

20.1.9 Reference Books

INTRODUCTION TO TEM:

A microscope is well-defined as an optical apparatus that uses an arrangement of lenses to yield magnified image of small objects. The discipline of examining small things using such an instrument is known as microscopy. It means untraceable to the eye if not assisted by a microscope. There are numerous types of microscopes and optical microscope is the most up-to-date and first manufactured microscope which utilizes light to image the sample.

- **Stereo-Microscope** uses a number of mechanisms that gather light and transmit the light track so that a magnified image of the observed object can be focused within a short distance.
- **Electron Microscope** are another category of microscopes which comprise of Transmission Electron Microscope and Scanning Electron Microscope.

The transmission electron microscope (**TEM**) was developed first, followed some years later by the scanning electron microscope (**SEM).**

20.1. TRANSMISSION EMISSION MICROSCOPE (TEM):

20.1.1 INTRODUCTION:

The Transmission Electron Microscope (TEM) produces a **two-dimensional (2D) image** of an ultra-thin section by capturing electrons that have passed through the specimen.

The degree of interaction between the electrons and the heavy metal stain influences the kinetic energy of the electrons, which are collected by a fluorescent plate. Transmission Electron Microscopy is particularly meant to study thin specimen which allows the incident electrons to traverse through after due interactions. These interactions are translated into signals which are magnified and focused on imaging device such as phosphor screen or a CCD camera (CCD-Charge Coupled Device) (**Figure 20.1**).

This technique allows visualization of samples on a molecular level, allowing topographical, morphological as well as compositional studies. TEM produce two-dimensional images of high-resolution of the order of nanometers and magnification ranging from 102 to 106 X, enabling to image planes and columns of atoms. The instrument can also be used to produce electron-diffraction patterns for crystalline studies.

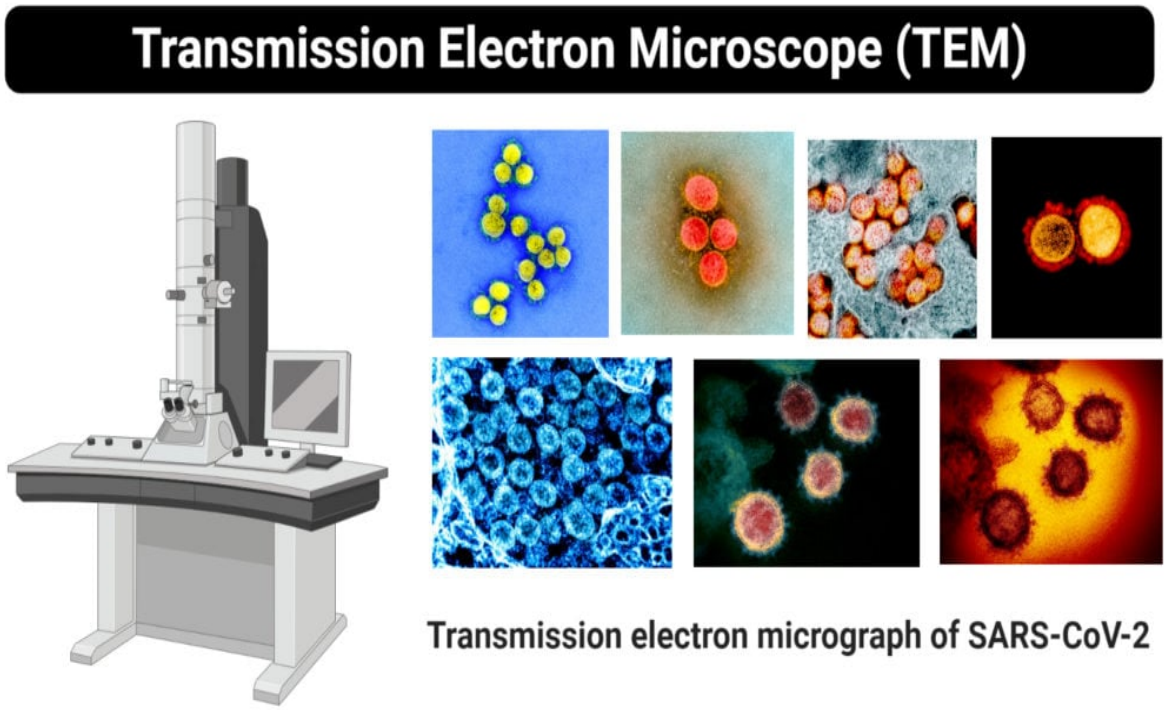

Figure 20.1: Instrumentation Image of TEM

20.1.2 INSTRUMENTATION OF TEM:

1. Illumination System:

- The illumination system comprises of an **electron source**, a thermionic gun which emits monochromatic beam of electrons.
- This beam of electrons possesses kinetic energy high enough to traverse through the thin areas of the specimen **(Figure 20.2)**.

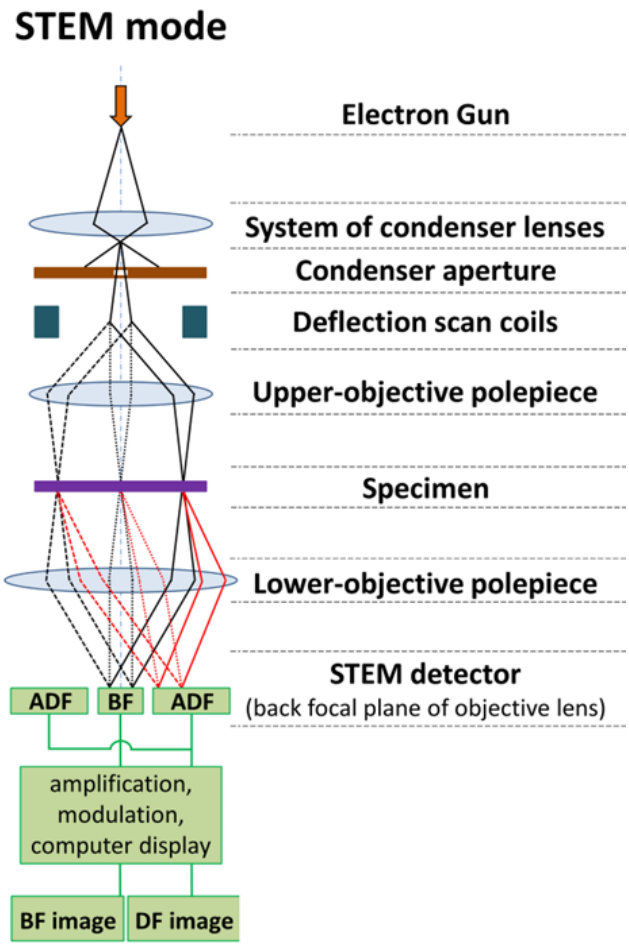

Figure 20.2: Components of TEM Imaging System

- Electron source, inside the gun is a pointed hair-pin shaped tungsten filament or a spike shaped lanthanum hexaboride source housed in an electron-accelerating chamber.
- The filament is connected to a high voltage supply (about 200-300 kV) which heats it up to about 2700 K, the temperature sufficient for thermionic emission of electrons from tungsten.
- Immediately after emission from the cathode, electrons are accelerated to their final kinetic energy by applying an electric field parallel to the optic axis.

2. The Condenser:

- The electron gun is followed by a condenser-lens system that converge the illumination and produces an almost parallel electron beam which is
- incident on the specimen in form of a spot.
- In order to achieve good contrast and wide range of magnification for viewing very small to larger objects in the specimen, most of the electrons that pass through the specimen should fall within a certain diameter corresponding to the screen.

3. The Stage:

- The specimen stage holds the specimen **perpendicular** to the optic axis.
- The specimen is placed on a ring-shaped grid and inserted through an airlock into the chamber which is evacuated before the specimen enters the TEM column.
- The sample is held stationary during imaging, although it can be displaced in three planes and tilted to an angle upto defined distance or degrees, with the help of holder adjustments.
- This helps to align a particular region of specimen into the path of the electron beam so that all possible regions of the specimen can be visualized.

4. Objective:

- It is an electromagnetic lens made of coils which encounter high excitation current and produce the magnetic field to focus the electrons.
- The focusing power of lens depends on its excitation and is susceptible to even slight variations in the current; therefore, highly stabilized current supply is required for accurate focusing of specimen.
- The coils of lens utilize high voltage and generate large amount of heat, thus are kept under insulation and are provided with thermal distributors.

5. Intermediate lens and Projector Lens:

- The intermediate lens produces pattern in the back focal plane of the objective lens which is used by the projector lens to form image on the screen.
- The projector lens produces a large image of several centimeters diameter, across the fluorescent screen.
- The projector lens has a focal length of a few mm which serves to minimize the image distortion occurring due to high angle scattering of electrons.

6. TEM Screen, Detectors and Image Recorder:

- A phosphor screen converts the diffraction pattern of electrons into visible light signals.
- The screen is made of fluorescing material Zinc Sulfide (ZnS). As it receives the electrons focused by projector lens, it proportionately emits light in visible Range.
- TEM is coupled with detectors which generate electrical signals proportional to the photon intensity.
- These signals are transformed into image which can be seen directly on the monitor or can be captured in a camera.

20.1.3 ADVANTAGES OF TEM:

- Transmission electron microscopy (TEM) offers high-resolution imaging and provides direct observation of information that can only be obtained statistically through other techniques such as x-ray and neutron crystallography.
- TEM can quantify the morphology, structure, composition, chemistry, bonding, and optical/electronic properties of nanostructures, interfaces, and defects in various materials and biological systems.

20.1.4 DISADVANTAGES OF TEM:

- One disadvantage is the potential for electron beam modification of the specimen during image acquisition, especially with the increase in beam current from using correctors.
- Another limitation is the electron beam damage that can occur during traditional TEM scanning, which can limit the resolution.
- Despite these limitations, TEM remains a powerful tool in nanomedical research, providing high-resolution images that contribute to the advancement of nanomedicine by studying the interactions between nanoparticulates and the biological environment.

20.1.5 APPLICATIONS OF TRANSMISSION ELECTRON MICROSCOPE (TEM):

TEM is used in a wide variety of fields From Biology, Microbiology, Nanotechnology, forensic studies, etc. Some of these applications include:

1) To visualize and study cell structures of bacteria, viruses, and fungi
2) To view bacteria flagella and plasmids
3) To view the shapes and sizes of microbial cell organelles
4) To study and differentiate between plant and animal cells.
5) It’s also used in nanotechnology to study nanoparticles such as ZnO nanoparticles
6) It is used to detect and identify fractures, damaged microparticles which further enable repair mechanisms of the particles.

20.1.6 LIMITATIONS OF TRANSMISSION ELECTRON MICROSCOPE (TEM):

1) Generally, the TEMs are very expensive to purchase
2) They are very big to handle.
3) The preparation of specimens to be viewed under the TEM is very tedious.
4) The use of chemical fixations, dehydrators, and embedment’s can cause the dangers of artefacts.
5) They are laborious to maintain.
6) It requires a constant inflow of voltage to operate.

7) They are extremely sensitive to vibrations and electro-magnetic movements hence they are used in isolated areas, where they are not exposed.
8) It produces monochromatic images, unless they use a fluorescent screen at the end of visualization.

20.1.7 SUMMARY:

- To know about the Microscopic studies.
- To study about basic Principle involved in TEM.
- To study about Instrumentation of TEM.
- To study about Advantages, Disadvantages and Applications of TEM.

20.1.8 SELF-ASSESSMENT QUESTIONS:

1) Write the basic principle and instrumentation of TEM.
2) Write the applications and disadvantages of TEM.
3) Write the basic principle and instrumentation of TEM.
4) Write the principle and applications of TEM.

20.1.9 REFERENCE BOOKS:

1) Introduction to Organic Laboratory Techniques-D.L. Pavia, G.M. Lampman, G.S. Kriz and R.G. Engel, Saunders College Pub. (NY).
2) Instrumental methods of Chemical Analysis by B.K. Sharma, Goel Publish House, Meerut.
3) Instrumental methods of Chemical Analysis by H. Kaur, Pragati Prakasan, Meerut.
4) A Hand Book of Instrumental Techniques for Analytical Chemistry- Ed-F.A. Settle, Prearson Edn.
5) Principles of Instrumental Analysis by D.A. Skoog, F.J. Holler and T.A. Nieman, Harcourt College Pub.

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LESSON-20.2

MICROSCOPIES STUDIES - TEM AND SEM

SCANNING ELECTRON MICROSCOPE (SEM)

20.2.0 OBJECTIVES:

After studying this lesson, you should be able to:

- To study about basic Principle involved in SEM.
- To study about Instrumentation of SEM.
- To study about Advantages, Disadvantages and Applications of SEM.
- To know about differentiation between SEM vs TEM.

STRUCTURE

SCANNING ELECTRON MICROSCOPE (SEM)

20.2.1 Introduction to SEM

20.2.2 Basic Principle

20.2.3 Parts of a Scanning Electron Microscope (SEM)

20.2.4 Disadvantages of Scanning Electron Microscopy

20.2.5 Applications of SEMs

20.2.6 Limitations of SEMS

20.2.7 SEM vs TEM

20.2.7 SEM vs TEM

20.2.8 Summary

20.2.9 Self-Assessment Questions

20.2.10 Reference Books

20.2.1 INTRODUCTION TO SEM:

Scanning Electron Microscope (SEM) is a type of electron microscope that scans surfaces of microorganisms that uses a beam of electrons moving at low energy to focus and scan specimens. The development of electron microscopes was due to the inefficiency of the wavelength of light microscopes. electron microscopes have very short wavelengths in comparison to the light microscope which enables better resolution power.

SEM requires low kinetic energy electrons which need not penetrate deep inside the sample, instead interact on the surface up to a few nm of the depth. The electron source is tungsten filament or a LaB_6 cathode housed inside an electron gun smaller than the one used in TEM. The electron beam is accelerated by a low voltage between 2 to 50 KV. The SEM column and sample chamber are at a moderate vacuum of about 10^{-5} to 10^{-7} Torr (10^{-3} to 10^{-4} Pa) to allow the electrons to travel freely from the electron beam source to the sample and

then to the detectors. The technique **does not require** to accelerate the electrons much, a strong magnetic field is not needed therefore magnetic lenses used are also smaller (**Figure 20.3**).

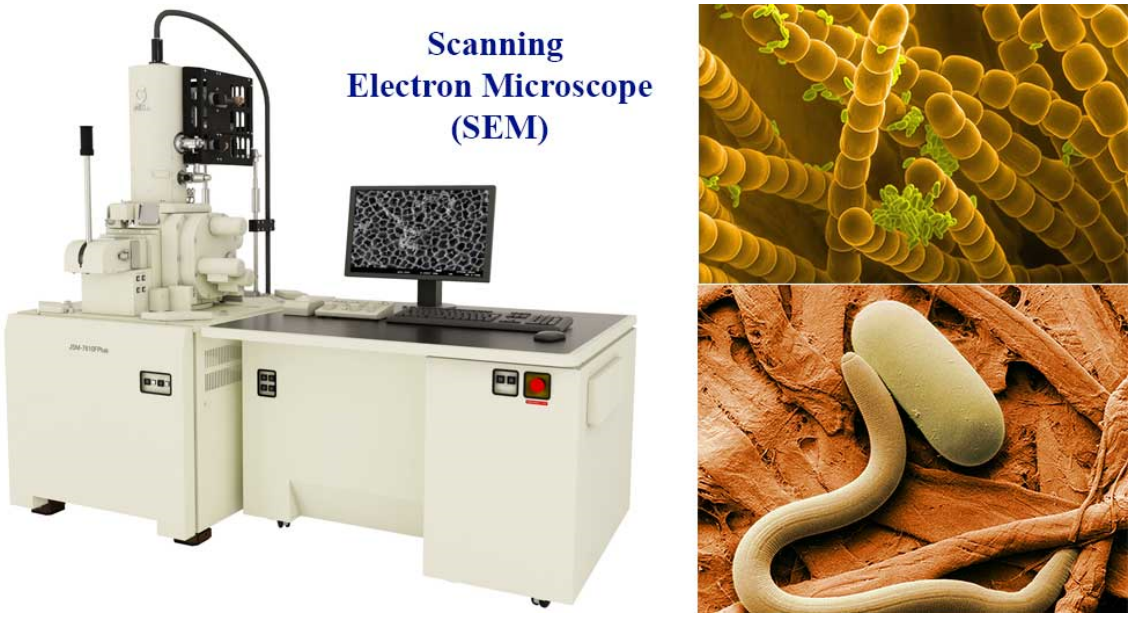

Figure 20.3: Instrumentation Image of SEM

20.2.2 BASIC PRINCIPLE:

Unlike the Transmission Electron Microscope which uses transmitted electrons, the scanning electron Microscope uses emitted electrons. The Scanning electron microscope works on the principle of applying kinetic energy to produce signals on the interaction of the electrons. These electrons are secondary electrons, backscattered electrons, and diffracted backscattered electrons which are used to view crystallized elements and photons. Secondary and backscattered electrons are used to produce an image. The secondary electrons are emitted from the specimen play the primary role of detecting the morphology and topography of the specimen while the backscattered electrons show contrast in the composition of the elements of the specimen (**Figure 20.4**).

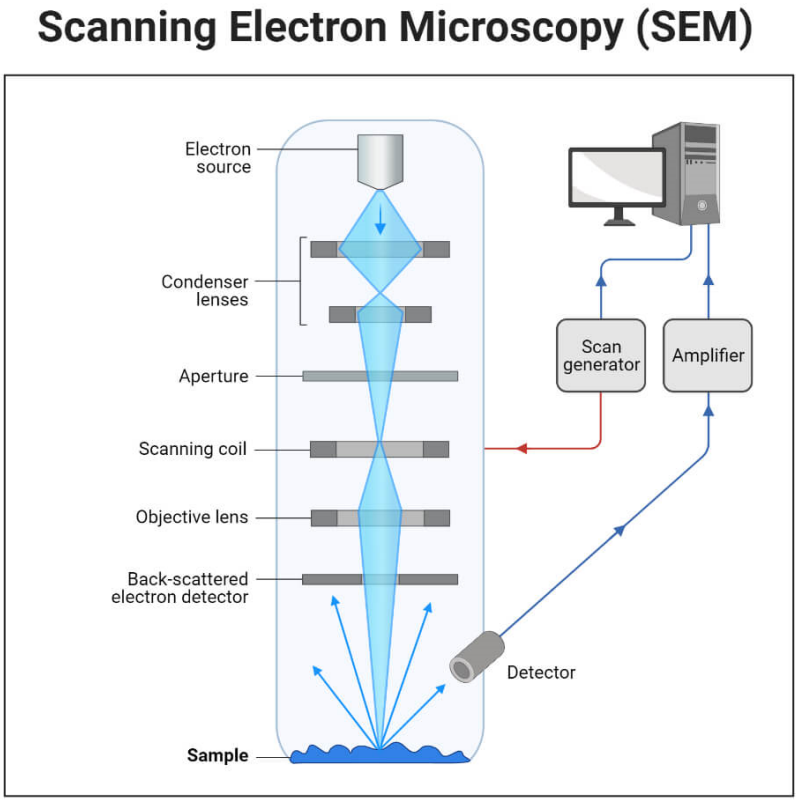

Figure 20.4: Working of Scanning Electron Microscope

- A two-stage lens system is placed below the electron gun which comprises of condenser lens and objective lens.
- The condenser lens narrows down the electron beam to a spot of about 0.5 to 5 nm diameter that scans the specimen.
- The final lens is an objective lens which focuses this very small probe on to the specimen.
- The specimen is mounted on a stage which holds it stably while allowing it to move in horizontal and vertical axes, tilt or rotate; so that all the areas of the specimen can be scanned.
- The beam now passes through pairs of scanning coils located near the end of the column, which deflect the beam in the x and y axes to perform a scan over the sample surface.
- The signals generated as a result of due interactions in form of SEs, BSEs etc. are detected for each position in the scanned area by an electron detector.
- Different signal intensities are displayed as patterns of varying brightness on a Cathode Ray Tube (CRT) corresponding to the morphology of the sample surface area scanned by the beam.
- The electron detectors predominantly used in SEM imaging are scintillator type also known as Everhart- Thornley detector meant for secondary electron imaging and solid-state detector for backscattered electrons.

20.2.3 PARTS OF A SCANNING ELECTRON MICROSCOPE (SEM):

The major components of the Scanning Electron Microscope include:

- **Electron Source** – This is where electrons are produced under thermal heat at a voltage of 1-40kV. the electrons condense into a beam that is used for the creation of an image and analysis. There are three types of electron sources that can be used i.e. Tungsten filament, Lanthanum hexaboride, and Field emission gun (FEG)
- **Lenses** – it has several condenser lenses that focus the beam of electrons from the source through the column forming a narrow beam of electrons that form a spot called a spot size.
- **Scanning Coil** – they are used to deflect the beam over the specimen surface.
- **Detector** – It's made up of several detectors that are able to differentiate the secondary electrons, backscattered electrons, and diffracted backscattered electrons. The functioning of the detectors highly depends on the voltage speed, the density of the specimen.
- The display device (data output devices)
- Power supply
- Vacuum system Advantages of Scanning Electron Microscopy

Here are some benefits of using SEM with EDS for materials characterization and failure analysis.

1. Resolution:

This test provides digital image resolution as low as *15* nanometers, providing instructive data for characterizing microstructures such as fracture, corrosion, grains, and grain boundaries.

2. Traceable Standard for Magnification:

Because all imaging is calibrated to a traceable standard, it's easy to apply analysis such coating thicknesses, grain size determinations, and particle sizing to saved images.

3. Chemical Analysis:

SEM with EDS provides qualitative elemental analysis, standardless quantitative analysis, x-ray line scans, and mapping. This data can be used to examine product defects, identify the elemental composition of foreign materials, assess the thickness of coatings, and determine grain and particle size.

20.2.4 DISADVANTAGES OF SCANNING ELECTRON MICROSCOPY:

While an excellent test for surface typography and chemical analysis, some samples are not a good fit for SEM with EDS. Here are a few reasons to consider different types of materials analysis.

1. Vacuum Environment:

In most cases, SEM samples must be solid and vacuum-compatible. However, higher pressures can be used for imaging of vacuum-sensitive samples that are nonconductive and volatile. For more information, read our comparison of conventional SEM, variable pressure SEM (VPSEM), and field emission SEM (FESEM).

2. Artifacts are Possible:

Samples that are strong insulators must be coated usually with gold or carbon-before testing. However, this process can result in artifacts. That said, preparation and analysis by a knowledgeable SEM testing lab ensures that these artifacts have minimal impact on testing results.

20.2.5 APPLICATIONS OF SEMs:

It is used in a variety of fields including Industrial uses, nanoscience studies, Biomedical studies, Microbiology

1) Used for spot chemical analysis in energy-Dispersive X-ray Spectroscopy.
2) Used in the analysis of cosmetic components which are very tiny in size.
3) Used to study the filament structures of microorganisms.
4) Used to study the topography of elements used in industries.

20.2.6 LIMITATIONS OF SEMs:

- They are very expensive to purchase
- They are bulky to carry
- They must be used in rooms that are free of vibrations and free of electromagnetic elements
- They must be maintained with a consistent voltage
- They should be maintained with access to cooling systems

20.2.7 SEM vs TEM:

Table 20.1: Summarizing key Differences Between SEM and TEM

Property	SEM	TEM
Signals Analysed	Backscattered electrons and secondary electrons	Transmitted electrons
Sample thickness	Any sample	< 100 nm for most materials
Accelerating voltage	~1-30 Kv	~30-300 kV
Max magnification	Up to 1-2 million times	50 million times or more

Fiels of view	Few µm to several mm`s	Few nm`s to several µm`s
Size of Instrument	Smaller, desktop models and floor model options	Larger and taller, takes up entire room
Cost	Less expansive	More expansive
Time to image	Fast	Slow
Spatial resolution	~1-2 nm	< 1 A^{o}
Image production	3D black and white images	Produces 2D black and white images
Field of view	Large	Limited

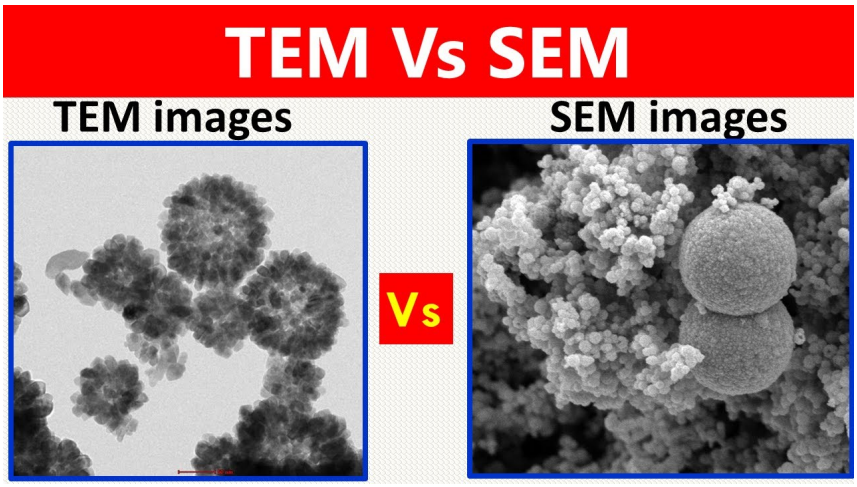

Figure 5: Differences Between SEM Vs TEM Images

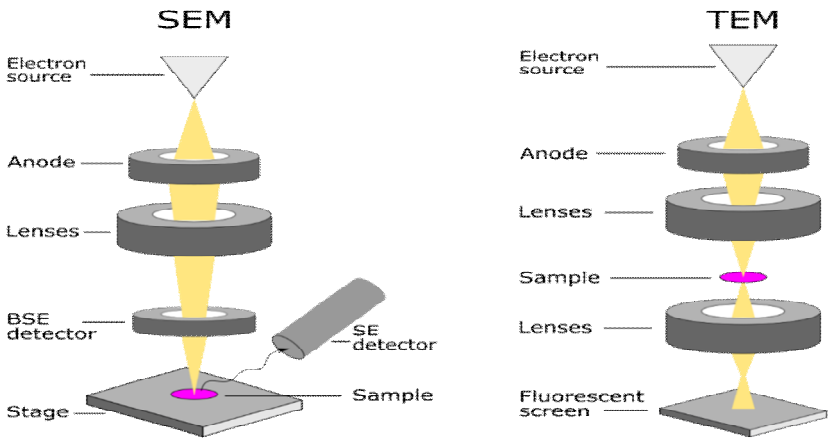

Figure 20.5: Differences Between SEM Vs TEM Instrumentation

20.2.8 SUMMARY:

- To study about basic Principle involved in SEM.
- To study about Instrumentation of SEM.
- To study about Advantages, Disadvantages and Applications of SEM.
- To know about differentiation between SEM vs TEM.

20.2.9 SELF-ASSESSMENT QUESTIONS:

1) Differentiate between SEM vs TEM.
2) Write the basic principle and instrumentation of SEM.
3) Write the applications and disadvantages of SEM.

20.2.10 REFERENCE BOOKS:

1) Introduction to Organic Laboratory Techniques-D.L. Pavia, G.M. Lampman, G.S. Kriz and R.G. Engel, Saunders College Pub. (NY).
2) Instrumental methods of Chemical Analysis by B.K. Sharma, Goel Publish House, Meerut.
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