



CHROMATOGRAPHY AND MASS SPECTROMETER

22.1 INTRODUCTION

We know that the biochemistry or biological chemistry deals with the study of molecules present in organisms. These molecules are called as biomolecules and they form the basic unit of every cell. These include carbohydrates, proteins, lipids and nucleic acids. To study the biomolecules and to know their function, they have to be obtained in purified form. Purification of the biomolecules includes many physical and chemical methods. This topic gives about two of the commonly used methods namely, chromatography and mass spectrometry. These methods deals with purification and separation of biomolecules namely, protein and nucleic acids.



OBJECTIVES

After reading this lesson, you will be able to:

- define the chromatography and mass spectrometry
- describe the principle and important types of chromatographic methods
- describe the principle and components of a mass spectrometer
- enlist types of mass spectrometer
- describe various uses of mass spectrometry

22.2 CHROMATOGRAPHY

When we have a mixture of colored small beads, it is easily separated by visual examination. The same holds true for many chemical molecules. In 1903,

Mikhail, a botanist (person studies plants) described the separation of leaf pigments (different colors) in solution by using solid adsorbents. He named this method of separation called chromatography. It comes from two Greek words:

chroma – colour

graphein – to write/detect

Modern separation methods are based on different types of chromatographic methods. The basic principle of any chromatography is due to presence of two phases:

- Mobile phase – substances to be separated are mixed with this fluid; it may be gas or liquid; it continues moves through the chromatographic instrument
- Stationary phase – it does not move; it is packed inside a column; it is a porous matrix that helps in separation of substances present in sample. It works on simple physical process called **adsorption**.



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22.2.1 Components of Chromatography

The equipment used for separation of mixture in a sample is called a chromatograph. The major components of a chromatograph:

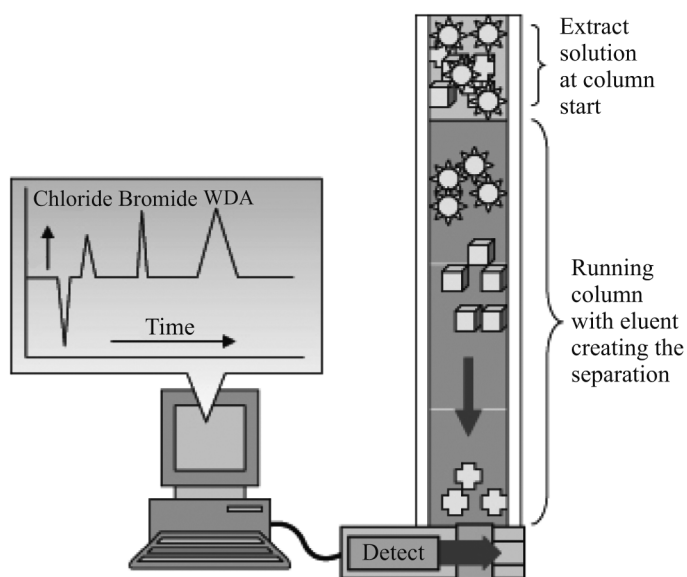


Fig. 22.1

- Mobile phase source – separate chambers or containers having either gas or liquid
- Analyte – sample to be separated
- Sample injection – chamber to inject the sample
- Separating column – having the stationary phase

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- Detector – identify the change in separation of a molecule; this calculates the time between injection and detection of a sample, which is called as **retention time**.
- Recorder and analyser – usually a computer attached to the chromatograph. This records the response of detector as a graph called chromatogram

22.2.2 Types

There are many types of chromatographic methods present today. Some of the important methods are described as follows:

- Gas chromatography
- Liquid chromatography
- Gel filtration chromatography
- Ion exchange chromatography
- Affinity chromatography
- Others- hydrophobic interaction chromatography (HIC), Isochromatic focusing (ICF), etc.

22.2.2.1 Gas Chromatography

The gas chromatography principle involves separation of the components of the sample due to separation in between the gaseous mobile phase and stationary phase (usually silica). The components separated into gas comes out first while other comes later. It detects compounds like fatty acids, essential organic solvents, flavored oils, etc.

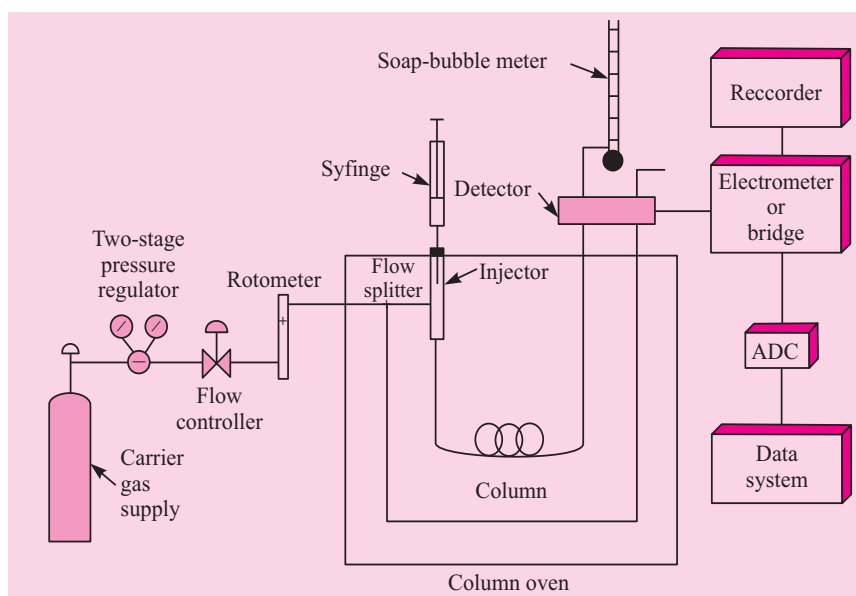


Fig. 22.2: Gas chromatography



In GC, a liquid sample is injected into the column. The GC column is usually coated with stationary phase and placed inside an oven chamber. The sample is vaporized as it passes the column which is more than its boiling point. The sample compounds are carried to column by gas (usually helium or nitrogen) and then to a detector. The detector signals a chart recorder for chromatogram (Fig.22.2)

22.2.2.2 Liquid Chromatography

It is separation technique where the mobile phase is a liquid. It can be performed in a column or a plane surface. This has been advanced to many types :

- HPLC – high performance liquid chromatography (Fig.22.3)
- FPLC – fast protein liquid chromatography

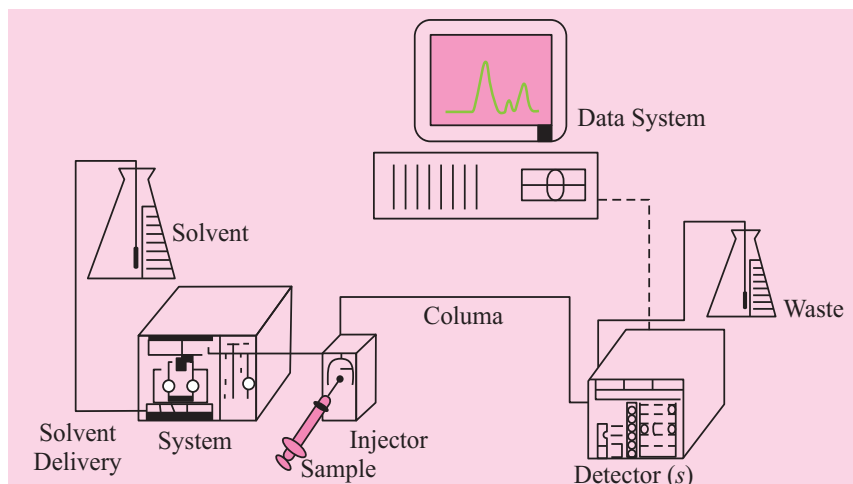


Fig. 22.3: HPLC

22.2.2.3 Gel Filtration Chromatography

This chromatographic technique is also known as size exclusion chromatography or molecular sieve chromatography. It involves the separation of molecules based on their molecular weight and size. (Fig.22.4) It involves:

- Mobile phase – liquid mixed with sample mixture
- Stationary phase – gel matrix containing a particular pore size to allow smaller molecules to easily pass through or vice versa.

It well suitable for separation of biomolecules that are sensitive to environmental conditions like temperature, pH, etc.



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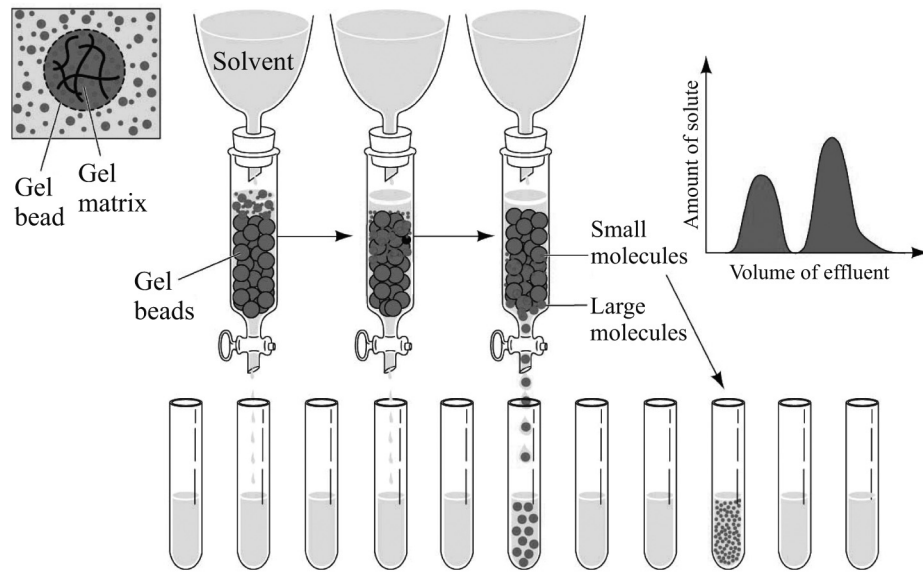


Fig. 22.4: Gel Filtration Chromatography

22.2.2.4 Ion Exchange Chromatography

Every chemical molecule that is capable of forming into ions (different charges – positive, **cations** and negative, **anions**) is separated by using this chromatography (Fig. 22.5). In this:

- Mobile phase – liquid
- Stationary phase – matrix of beads with either positive charge (cationic) to separate anionic sample or negative charge (anionic) to separate cationic sample.

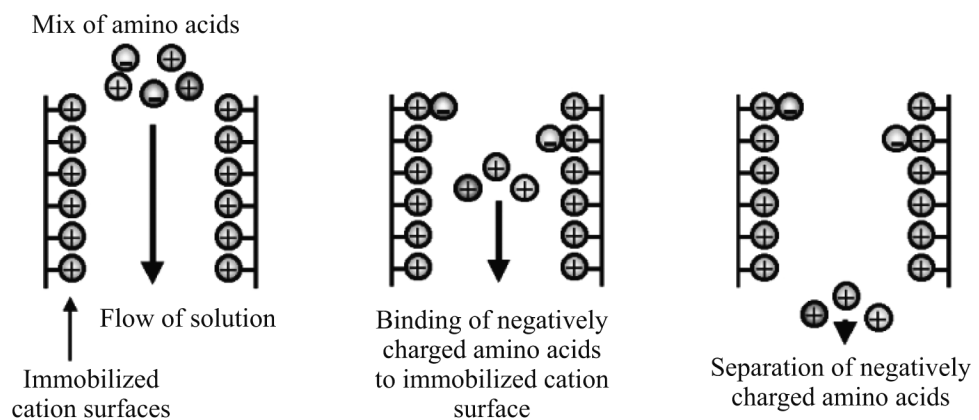


Fig. 22.5: Ion Exchange Chromatography

It is used to separate charged proteins and peptides in a given sample.

22.2.2.5 Affinity Chromatography

Affinity chromatography separates proteins on the basis of a specific interaction between the molecules in the sample with a compound called ligand in the column (Fig.22.6). Some of the ligands used in matrix are:

- enzymes
- antibody
- metal ions like Nickel, etc.

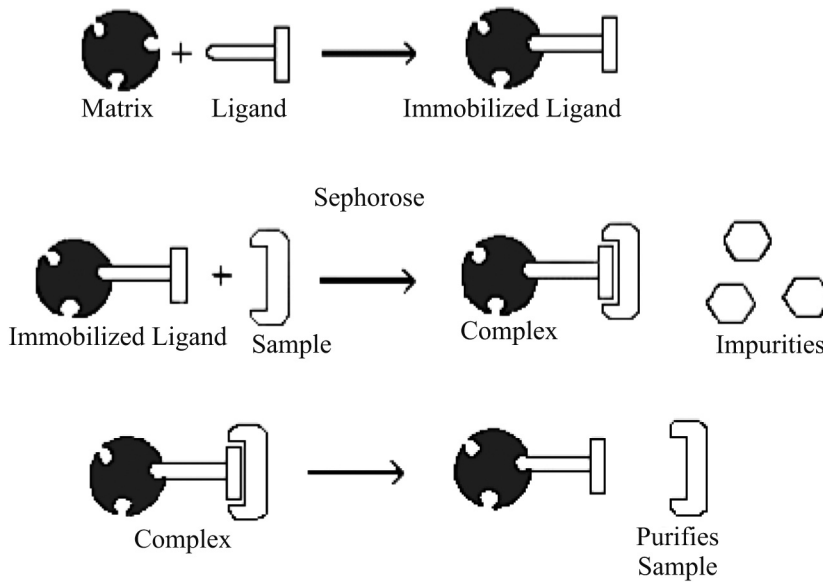


Fig. 22.6: Affinity Chromatography

This technique is highly specific and is used mostly for purification of proteins and peptides.

22.3 MASS SPECTROMETRY

The mass spectrometer is an instrument that measures the masses and relative concentrations of atoms and molecules. It makes use of the basic magnetic force or velocity on a moving charged particle.

22.3.1 Basic Principle

Suppose you had a cannonball travelling past you and you wanted to deflect (change of direction) it. All you've got is a jet of water from a hose-pipe. But, it's not going to make a lot of difference! Because the cannonball is so heavy, it will hardly be deflected at all from its original course.



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If suppose instead of water, you try to deflect the table tennis ball travelling at same speed using jet of water. Because the ball is so light, it will be deflected easily.

Thus from the above examples, we can understand that amount of deflection depends on the weight (mass) of the object. The same principle holds true for atom-sized molecules.

22.3.1.1 Working Principle

“The technique for studying the masses of atoms or molecules or fragments of molecules separated by their mass-to-charge ratio (m/z)”.

22.3.2 Components

There are many different kinds of mass spectrometers, but all use magnetic and/or electric fields to exert forces on the charged particles produced from the chemicals to be analyzed (Fig. 22.7). A basic mass spectrometer consists of three parts:

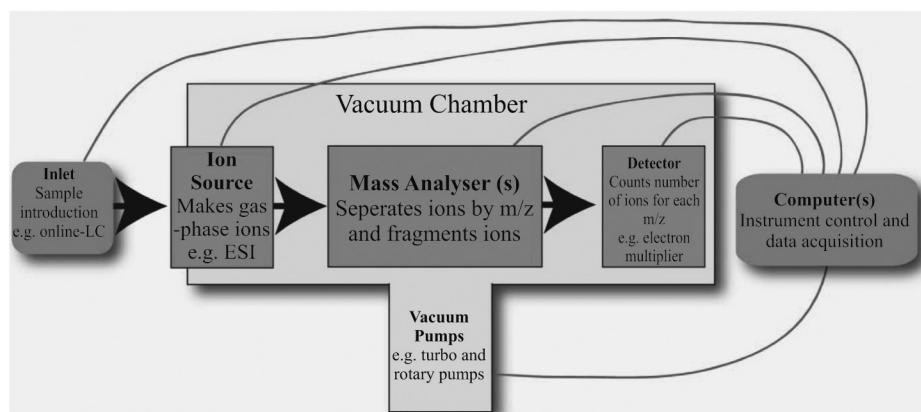


Fig. 22.7: Mass Spectrometry - components

1. **Source** - in which ions are produced from the chemical substances to be analyzed.
2. **Analyser** - in which ions are separated according to mass.
3. **Detector** - which produces a signal from the separated ions.

A magnetic field or electric field separates ions according to their momentum (mass x velocity)

22.3.3 Types of Mass Spectrometry

There are many different kinds of mass spectrometers based on the type of ion source, mass analyzer and detector used. Let us see the various types of ion source and mass analyzers:

22.3.3.1 ESI – TQ Mass Spectrometer

The basic workflow of ESI tandem MS is as follows:

- the proteins pass through the source become ions;
- then the ions pass through the first analyzer and some specific ions are selected;
- then these selected ions are broken up by a procedure called collision-induced dissociation (CID);
- finally the second analyzer is used to catch the ions produced after CID.

ESI – Electro Spray Ionization – helps in ionizing all molecules of a given sample (Fig. 22. 8)

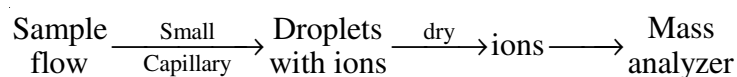


Fig. 22.8: Work flow of ESI ion source

Mass Analyzer – it may be of:

- Triple Quadrupole – consists of three single quadrupole mass analyzers Q1, Q2 and Q3.
- Ion Trap – traps specific molecules/ions with a particular m/z ratio and leaves all other to pass through; voltage is then suddenly increased leading to colliding of molecules/ions and then detection (Fig. 22.9)

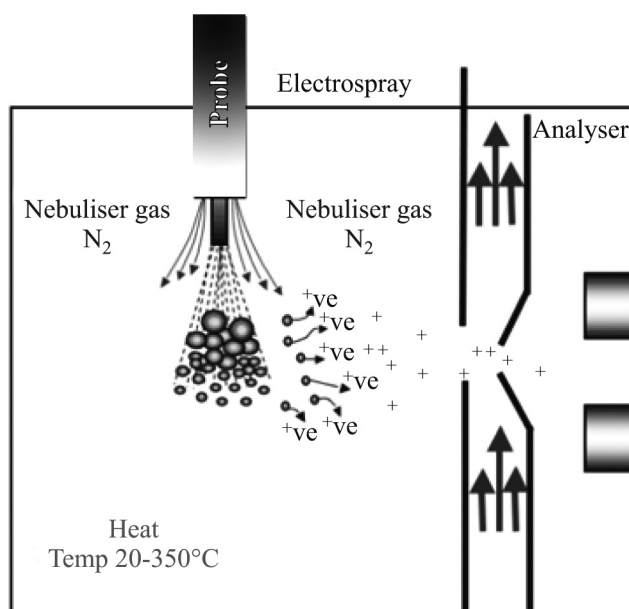


Fig. 22.9: ESI Tandem Mass Spectrometer



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22.3.3.2 MALDI TOF Mass Spectrometer

MALDI – Matrix Assisted Laser Desorption and Ionisation, is a soft ionization technique used for analysis of biomolecules (Fig.22.10)

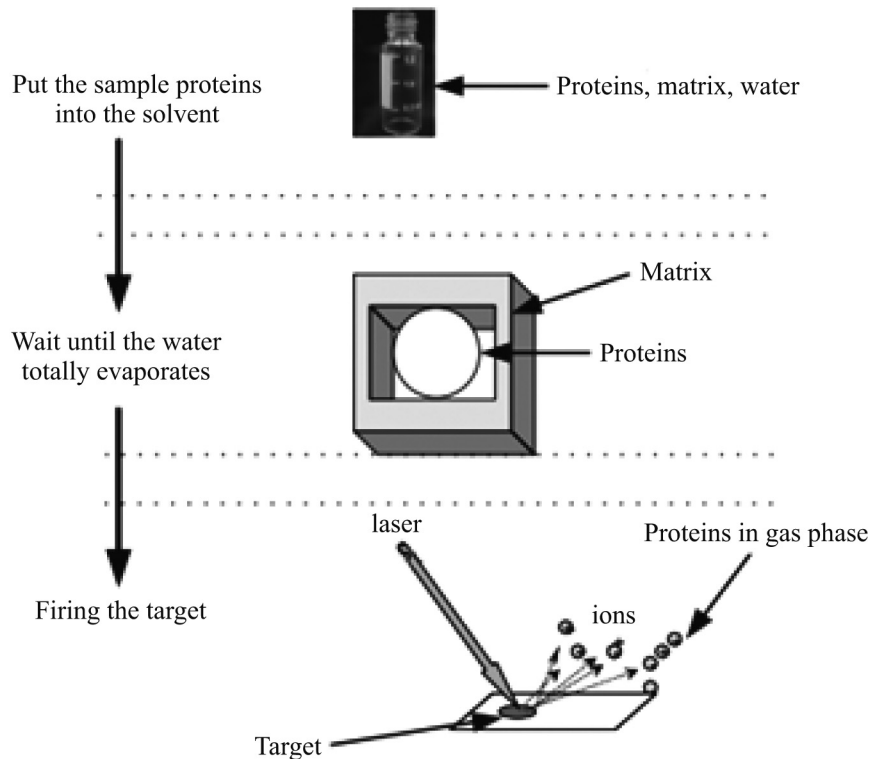


Fig. 22.10: Ion source of MALDI

TOF – Time Off Flight analyzer - An ion of known electrical charge and unknown mass enters a mass spectrometer and is accelerated by an electrical field of known strength. This acceleration results in any given ion having the same kinetic energy as any other ion given that they all have the same charge. The velocity of the ion will depend however on the m/z ratio (Fig.22.11).

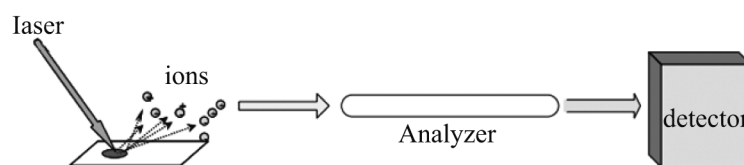


Fig. 22.11: TOF Analyzer and Detector

The result given by the detector is in the form of a graph called as a mass spectrum based on the different molecules present in the sample (Fig. 22.12).

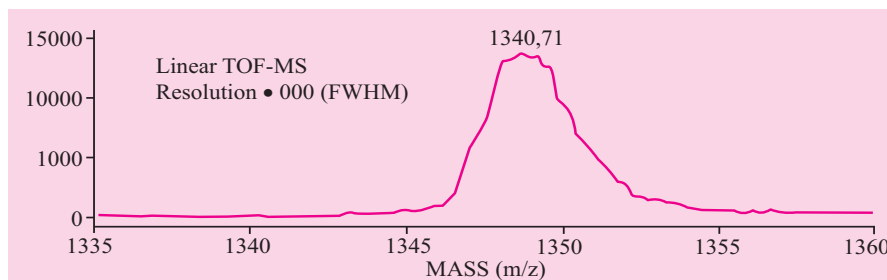


Fig. 22.12: Mass Spectrum



Notes

22.3.4 Uses

- Helps in identification of similar fragments of a molecule
- It can easily combine different ion source and mass analyzers.
- Has high resolution, friendly and robust; highly sensitive
- It is used for all kinds of chemical analyses, ranging from environmental analysis of petroleum products, trace metals and biological materials.



INTEXT QUESTIONS 22.1

1. Phases of chromatography are &
2. Types of liquid chromatography are &
3. Gel filtration chromatography separates molecules based on &
4. The instrument that measures the masses and relative concentration of atom and molecules is



WHAT HAVE YOU LEARNT

- Among the different methods to separate large and complex biomolecules, chromatography and mass spectrometry are important today.
- Each method can be used either individually or together to find out the composition of a large molecule.
- Based on the different properties and characters of molecules, differences in chromatography and mass spectrometry are discussed in the text.
- Finally, the need to study and importance of each of the methods are analyzed.

MODULE

Biochemistry

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TERMINAL QUESTIONS

1. Why is it necessary to separate and study molecules?
2. Define chromatography and the principle on which it works.
3. Discuss the major components of a chromatographic system.
4. What are the different chromatographic techniques?
5. Explain the basic principle of mass spectrometry.
6. Discuss the difference between TQ and TOF mass analyzers.



ANSWERS TO INTEXT QUESTIONS

22.1

1. Mobile and Stationary
2. High performance & Fast Protein
3. Molecular weight and Size
4. Mass Spectrometry