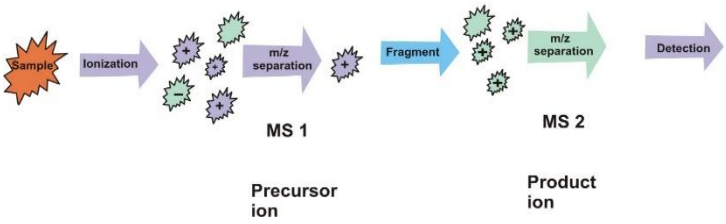


UNIT 7

MASS SPECTROMETRY

Structure

7.1 INTRODUCTION

You have earlier acquired the knowledge about the isolation and separation of proteins in block-I of this course. Now, you are going to learn about characterization of proteins using mass-spectrometry, tandem mass spectrometry and solid phase peptide synthesis. Mass spectrometry is the method of choice at present for fast and accurate characterization of proteins.

This technique has advantages over other methods in terms of obtaining molecular masses and for deriving their primary structures (amino acid sequence information). You may understand the importance of the protein characterization because protein is the major component of the cell/tissue and their characterization rely on our present knowledge of their structures, stabilities and performance under a variety of physical and chemical conditions. Tandem-mass spectrometry is also used for structural elucidation. Solid-phase synthesis is a common technique for peptide synthesis. Typically, peptides are synthesized from the carbonyl group side (C-terminus) to amino group side (N-terminus) of the amino acid chain in this method, while peptides are biologically synthesized in the opposite direction in cells. In this unit, we will learn the principle of mass spectrometry, components of mass spectrometer and its application. We will also learn the tandem-mass spectrometry and solid phase peptide synthesis.

Expected Learning Outcomes

After studying this unit, you should be able to:

- define the principle of mass spectrometry;
- explain the application of mass spectrometry;
- describe the principle of tandem mass spectrometry; and
- list the application of solid phase synthesis and its application.

7.2 MASS SPECTROMETRY

7.2.1 Mass Spectrometry: An Introduction

Mass spectrometry is a technique and applied to determine the chemical composition and the structure of a molecule. This is carried out by converting the molecule into ionized state and measures their mass to charge ratio.

History of Mass Spectrometry

The story of mass spectrometry was started from the inventive work of English scientist J.J. Thomson. He performed the improvement in the work of Wilhelm Wien and developed the first canal rays based mass spectrometry. Application of mass spectrometry was started for analyzing the amino acid in 1958. Moreover, Carl-Ove Andersson was the first who emphasize the major fragment ions detected in the ionization of methyl esters. Original models of mass spectrometry were developed by Arthur Jeffrey Dempster (1918) and F.W. Aston (1919) were given the original models of mass spectrometry.

Instead of that, the enormous research work of Hans Dehmelt (1950) and Wolfgang Paul (1960) appeared in the invention of the ion trap technique and also awarded the Nobel Prize of Physics in 1989.

A novel method for mass spectrometric analyses of biological macromolecules was developed by John Bennett and Koichi Tanaka, they received Nobel Prize in Chemistry in 2002.

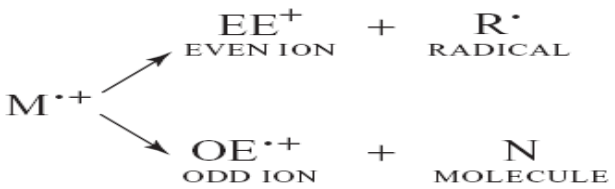
7.2.2 Principle of Mass Spectrometry

Mass spectrometry has diverse principles to some extent as compared to the other spectroscopic techniques. The charged particle passing through a magnetic field is deflected along a circular path on a radius that is proportional to the mass to charge ratio [m (mass) /e (charge)]. A high energy beam of electrons is used to displace an electron from the molecule to form a radical cation known as the molecular ion. Further, this molecular ion is fragmented and converted into smaller ions. The collected ions are focused into a beam and accelerated into the magnetic field and deflected along circular paths according to the masses of the ions. The ions can be focused on the detector and recorded by adjusting the magnetic field.

The most significant footstep in the mass spectrometric examination of a molecule is the construction of gas phase ions. The most excellent example is electronic ionization:

$$M + e^- \longrightarrow M^{\bullet +} + 2e^-$$

This molecular ion typically undertakes fragmentations. Because it is a radical cation with an odd number of electrons, it can fragment to provide moreover a radical or an ion with an even number of electrons, or a molecule and a new radical cation. There is a need of the explanation for the important differences between these two types of ions. In this regard, these ions are presented in a suitable form:



Diverse chemical properties are linked to these two kinds of ions. Each and every primary product ion generated from the molecular ion can, in turn, continue degeneration, and subsequently.

Separation of entire ions is achieved by mass spectrometer on the ground of their mass-to-charge ratio, and is distinguished in portion to their abundance.

Mass Spectrometry: Mechanisms of Action (How it Works)

The entire mass spectrometry techniques contain an analogous component. Types of mass spectrometry are depending on the difference in nature of these components. But, the mechanistic function of all the mass spectrometry tools are carried out in a unique mode. Mass spectrometer needs to perform three functions:

1. **Generation of ions-** Ions may be produced either by removing an electron from the molecule to produce a positively charged cation or by

adding an electron to form an anion. Generation of a cation and anion from a molecule M is represented by Fig. 7.1.

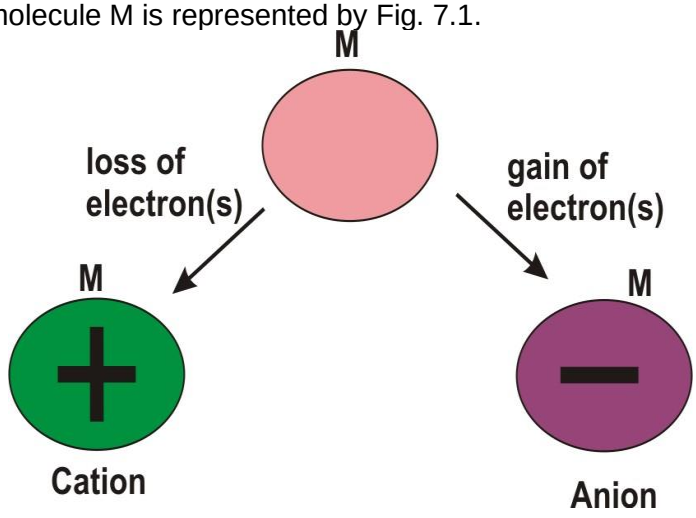


Fig. 7.1: Generation of cation with loss of electron and anion with gain of electron from a molecule (M).

You know very well about the above process, electron (e^-) carry the negative charge. When negative charge is removed, the molecule carries the positive charge and known as positive ion as cation. Similarly, if a molecule adopts the electron, it converts into a negative charged ion or anion.

2. **Separation of ions- The** ions (chiefly cations) are separated according to mass to charge ratio (m/z) where M ratio mass and Z charge). Separation of ions is performed by applying acceleration through an electric field and deflection *via* electromagnet.

3. **Detection of ions-** Qualitative (identification of particular ion) and quantitative (quantity or amount of particular ion) analysis are performed in the separated population of ions. After detection of ions, obtained data is collected for processing and ultimate result is going to produce on actionable information. Fig. 7.2, represented the outline of all the three functions of the mass spectrometer.

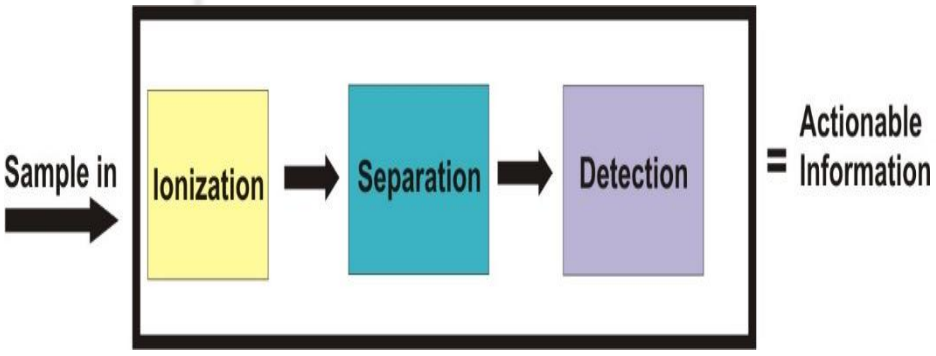


Fig. 7.2: All the three functions (ionization, separation and detection) of mass spectrometer connected to each other and produce the ultimate actionable information.

7.2.3 Components of Mass Spectrometer

Mass spectrometer contains three major components. These are ion source, mass analyser and detector. The following figure (Fig. 7.3) represented these components:

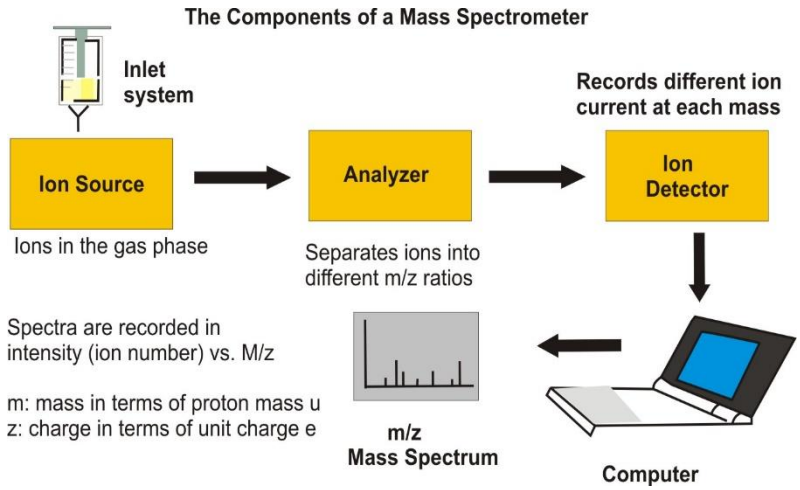


Fig. 7.3: Ion source, mass analyser and detector are the three components of mass spectrometer.

(1) Ion source

Samples are ionized by ion source of the mass spectrometer. There are various methods available to produce the molecular ions. There are the electron ionization (EI), the chemical ionization (CI), the photoionization (PI) and electrospray. In all these electrospray method and photoionization are most commonly used in protein analysis. These methods are described below:

Electrospray ionization (ESI): Electrospray ionization (ESI): Electrosprary ionization of a molecule is achieved by grounding a strong electric field (4kV) to a liquid transitory in the course of a capillary tube with a weak flux. In the next step, solubization process is carried out at 1atm pressure by nitrogen (N_2) gas flow or gentle capillary heating at 100^o C- 300^o C. Ions are mostly produced in solution before the process of desorption. This method is advantageous over other methods because this is nicely applied on both small and large molecules and produces mostly multiple protonated ions. This method has a very low energy transfer process (Fig. 7.4).

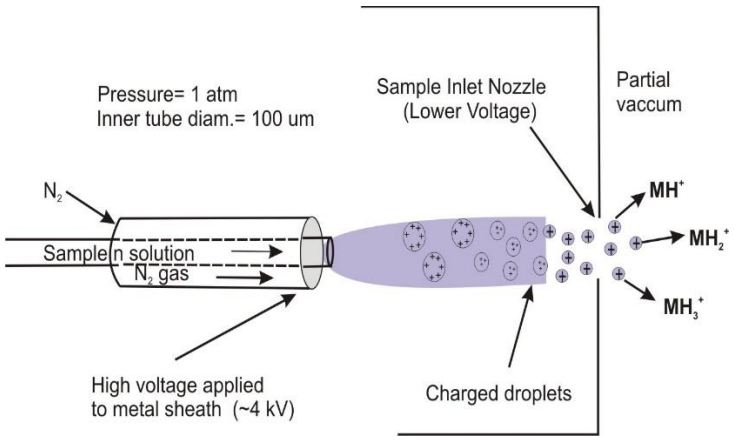


Fig. 7.4: Electrospray ionization process perform the ionization of the sample molecule.

Photoionization method

In this method, the sample (M) is mixed with matrix (X) and dried on plate. The flash of laser light is applied on the matrix molecule. Then the matrix molecules are ionized. Further, the sample molecule (M) is ionized by proton transfer of matrix molecule (X) (Fig. 7.5).

This is carried out in following reaction:

$$[XH^+ + M \longrightarrow MH^+ + X]$$

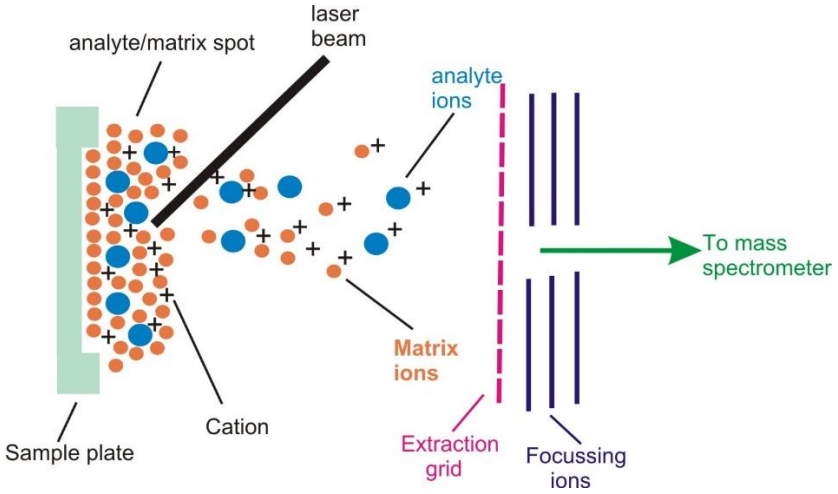


Fig. 7.5: Photoionization process perform the ionization of the sample molecule.

(2) Mass analyser

After completing the ionization process, there is a need of their separation. The physical possession of ions that is calculated by a mass analyser is their mass-to-charge ratio (m/z) relatively as compared to their mass unaided. Accordingly, it should be exposed that for numerous charged ions the perceptible m/z values are fractional pieces of their actual masses.

Due to the accessibility of a huge variety of sources, numerous kinds of mass analyser have been developed. Indeed, the separation of ions grounded on their mass-to-charge ratio afterward recognized dissimilar principles (Table-7.1). The entire kinds of mass analyser are linked with static or dynamic electric and magnetic fields. The majority of the ground distinctions between the several widespread kinds of mass analyser lie in the approach in which such fields are utilized to get isolation.

Table 7.1: Types of analysers used in mass spectrometry

Types of analyser	Symbol	Principle of separation
Electric sector	E or ESA	Kinetic energy
Magnetic sector	B	Momentum
Quadrupole	Q	*m/z* (trajectory stability)
Ion trap	IT	*m/z* (resonance frequency)
Time-of-flight	TOF	Velocity (flight time)
Fourier transform ion cyclotron resonance	FTICR	*m/z* (resonance frequency)
Fourier transform orbitrap	FT-OT	*m/z* (resonance frequency)

(3) Detectors

The ions are then identified and converted into an utilizable signal by a detector. Detectors are able to produce from the incident ions an electric current that is proportional to their abundance.

Numerous kinds of detectors currently exist. The option of the detector depends on the blueprint of the apparatus and the investigative applications that will be achieved. A small number of detectors are grounded in the measurement of direct charge current. Electro-optical ion detectors are ground with the kinetic energy transmit of incident ions by collision with a surface that in turn produces secondary electrons, which are afterward enlarged to provide an electronic current.

Extra detectors, for example, photographic plates, image current detectors or array detectors, have the ability to calculate multiple masses and recognize the influx of the entire ions simultaneously along a plane. These detectors are efficient for the majority of the applications in mass spectrometry.

7.2.4 Mass Spectrometer: The Whole Instrument

Now, you are aware with the components of the mass spectrometer. Now, all the components combined together and formed the mass spectrometry instrument. The following figure represent (Fig. 7.6) the schematic diagram of mass spectrometer:

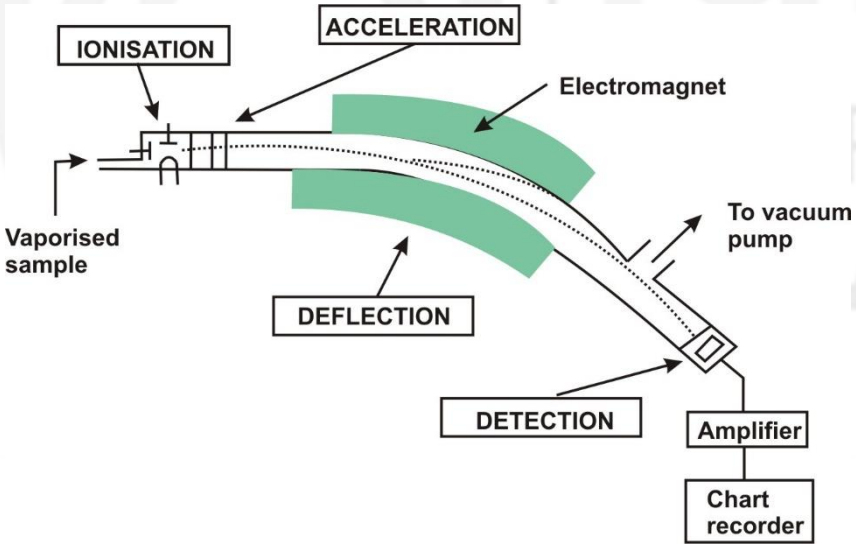


Fig. 7.6: Schematic diagram of mass spectrometer.

There are four key points in the process for mass spectrometer. These are ionization, acceleration, deflection and detection. The molecule is attacked by electrons which come from a heated filament. The electrons are flowing in a stream between the cathode and anode. This stream of electrons passes through the molecule and generated the ions. Acceleration is a easy step where the ions are placed between a set of charges parallel plates. The ions will then be repelled by one plate and attracted to the other. There is a slit cut in the plate which the ions are attracted and the force of attraction and repulsion forces the ions pass through the slit at an accelerated rate. The

speed of acceleration can be adjusted by changing the charge on the plates. Ions are deflected by the magnetic field surrounding the instrument. The amount of deflection depends on the mass and charge of the ions. The heavier ions and ions with a positive charge of two or more, are deflected the least. The lightest ions and ions with single positive charge are deflected the most. The ions at the correct mass and charge travel to the detector. The mass to charge ratio (m/z) is determined from the ion that hits the detector. The ion stream reached the detector to the hit a wire. On hitting the wire, they become neutralized by an electron jumping from the metal wire to the ion. The amplifier picks up on this current being created between the wire and the ion and amplifies the signal being detected. The computer picks up on this and converts it to mass/charge ratio and a spectrum is produced.

7.2.5 Applications

Mass spectrometry is applied in several fields, for example forensic toxicology, metabolomics, proteomics, pharma and clinical research. Precise applications of mass spectrometry include drug testing and discovery, food contamination detection, pesticide residue analysis, isotope ratio determination, protein identification, and carbon dating. Here, the several considerable applications are scheduled below:

(1) **In Proteomics** - Characterization of proteins and protein complexes, sequencing of peptides, and identification of post-translational modifications.

(2) **In Metabolomics** - Cancer screening and diagnosis, global metabolic fingerprinting analysis, biomarker discovery and profiling, biofuels generation and use, lipidomics studies, and metabolic disorder profiling.

(3) **In Environmental analysis** – Testing potable water testing, pesticide screening and quantification, soil contamination assessment, carbon dioxide and pollution monitoring, and trace elemental analysis of heavy metals leaching.

(4) **Pharmaceutical analysis** - Drug discovery and absorption, distribution, metabolism, and elimination (ADME) studies, pharmacokinetic and pharmacodynamic analyses, metabolite screening, and preclinical development.

(5) **Forensic analysis** - Analysis of evidence (e.g., fibers in carpet, polymers in paint), arson investigation (e.g., fire accelerant), confirmation of drug abuse, and identification of explosive residues (bombing investigation).

(6) **Clinical applications** - Phase studies of Clinical drug development, clinical tests, disease screening, drug therapy monitoring, analysis of peptides used for diagnostic testing, and identification of infectious agents for targeted therapies.

SAQ 1

1. Define the mass spectrometry?
2. Who invented the mass spectrometry?

3. What are the three functions of mass spectrometry?

4. Fill in the blanks:

i) The charged particle passing through a…… is deflected along a circular path on a radius that is proportional to ……

ii) The primary step in the mass spectrometric investigation of a molecule is the production of .....

iii) The physical property of ions that is measured by a mass analyser is their mass-to-charge ratio (*m/z*) rather than…… alone.

iv) All types of mass analyser are associated with static or dynamic……and ……. fields.

7.3 TANDEM MASS SPECTROMETRY

As far we have discussed about of mass spectrometry principle basic instrumentation and applications. In this section of unit, we shall be studying the details of Tandem Mass spectrometry that includes principle, instrument and applications.

7.3.1 Introduction

Tandem mass spectrometry (MS/MS) can be reflected as one broad-spectrum method connecting at least two steps of mass analysis, either in conjunction with a dissociation process or in a chemical reaction that grounds an alteration in the mass or charge of an ion. Tandem mass spectrometry is also known as MS/MS or MS^2.

J. J. Thomson is considered as a father of mass spectrometry. He is also considered the forefather of tandem mass spectrometry. Thomson constructed the first MS/MS apparatus, which contained in a sequential collection of two magnets, with the field on one magnet slanting perpendicular to the other. Tandem mass spectrometry can be recognized two kinds of methods: in space by the coupling of two mass spectrometers, or in time by a suitable series of events in an ion storage device.

7.3.2 Principle of Tandem MS

This accordingly leads to two main groups of apparatus that allow for tandem mass spectrometry experiments: tandem mass spectrometers in space or in time. This consequently directs to two major groups of instruments that permit for tandem mass spectrometry experiments: tandem mass spectrometers in space or in time (Fig. 7.7).

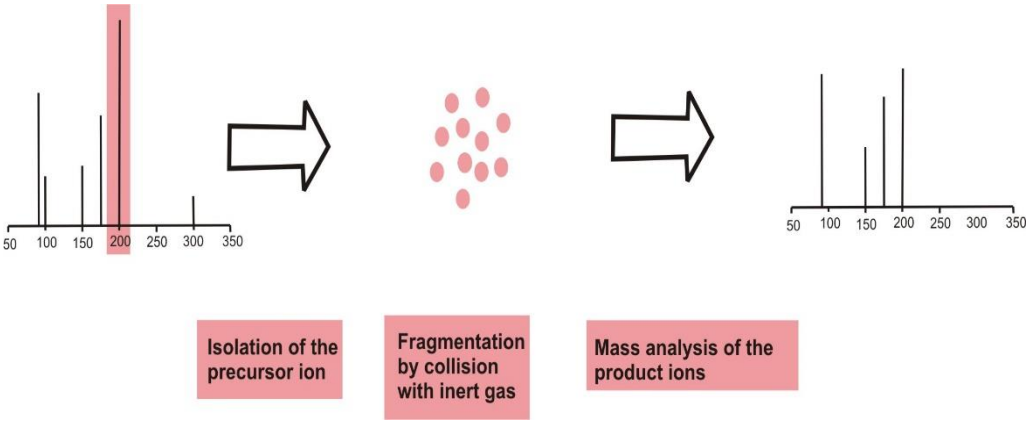


Fig. 7.7: Principle of MS/MS is represented. A precursor ion is separated by the initial mass analyser, fragmented by collision with an inert gas and the product ions are analysed by the second mass spectrometer.

In the several usual tandem mass spectrometry research the first analyser is utilized to isolate the precursor ion (mp+), which then continues fragmentation to obtain product ions (mf+) and neutral fragments (mn). Further, these are analysed by a second mass analyser. This reaction is described in following equation:

$$m_p^+ \rightarrow m_f^+ + m_n$$

7.3.3 Instrumentation of Tandem Mass Spectrometer

Simple and schematic diagram of tandem mass spectrometer is represented in Fig.7.8.

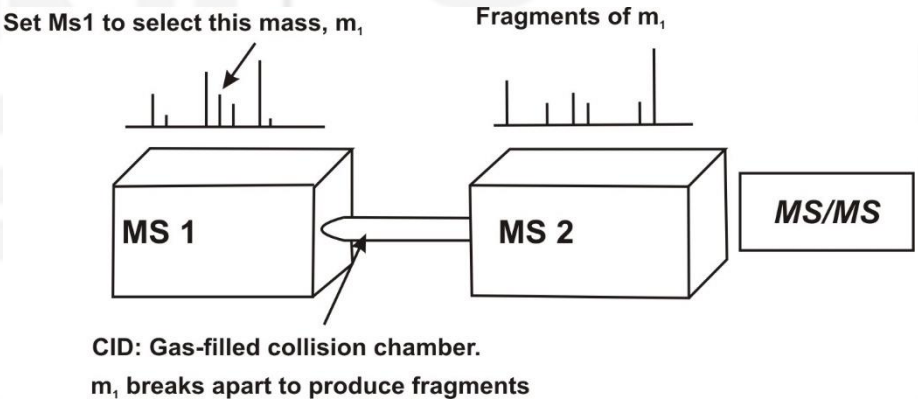


Fig. 7.8: Schematic diagram of tandem mass spectrometer.

Tandem mass spectrometer is a combination of two mass spectrometers. The first mass spectrometer is applied to select a precursor mass that is characteristics of a given analyte in a mixture. The mass-selected ions pass through an area where they are activated in several ways. This activation is responsible to produce fragment or product ions. This is generally performed by colliding the ions with a neutral gas in a process called collisional activation (CA) or collision-induced dissociation (CID). The second mass spectrometer is applied to isolate the fragment ions according to mass. The resulting “MS / MS” spectrum consists only of product ions from the selected precursors.

7.3.4 Working Mechanism of Tandem Mass Spectrometry

The samples are ionized to generate a mixture of ions, precursor ions of a specific mass-to-charge ratio (m/z) is selected (MS1) and then fragmented (MS2) to generate a product ion for detection. The sequence of selection-fragmentation-detection can be utilized to produce the first-generation product ions. Selected product ions generated in MS2 can be further fragmented to produce another group of product ion (MS3) and so on (Fig. 7.9). Numerous phases of mass analysis, isolation can be achieved by individual mass spectrometer elements separated in space or utilizing a solitary mass spectrometer with the MS steps separated in time.

i) Tandem mass spectrometry in space

In tandem mass spectrometry in space, the divider elements are physically alienated and different. But there is a physical linked between the elements to persist high vacuum. These elements are sectors, transmission quadrupole and time-of- flight. They can perform as together, mass analyzers and collision chambers throughout the utilization of multiple quadrupoles.

Usually, applicable mass analyzers are quadrupole mass analyzer, radio frequency collision quadrupole, time-of-flight mass analyzer, magnetic sector and electric sector.

ii) Tandem mass spectrometry in time

In the situation of tandem mass spectrometry in time, the separation is performed through ions ensnared in the identical place, with numerous separation steps taking place over time. Fourier transform ion cyclotron resonance (FTICR) instrument can be utilized for such analysis.

Fragmentation

Several approaches are utilized to fragment the ions and these can result in miscellaneous kinds of fragmentation and consequently divergent information is obtained about the structure and composition of the molecule.

Post-source fragmentation is generally used in a tandem mass spectrometry experiment. Collision-induced dissociation (CID) is also considered as Collisionally activated dissociation (CAD), engages the collision of an ion with a neutral atom or molecule in the gas phase and subsequent dissociation of the ion.

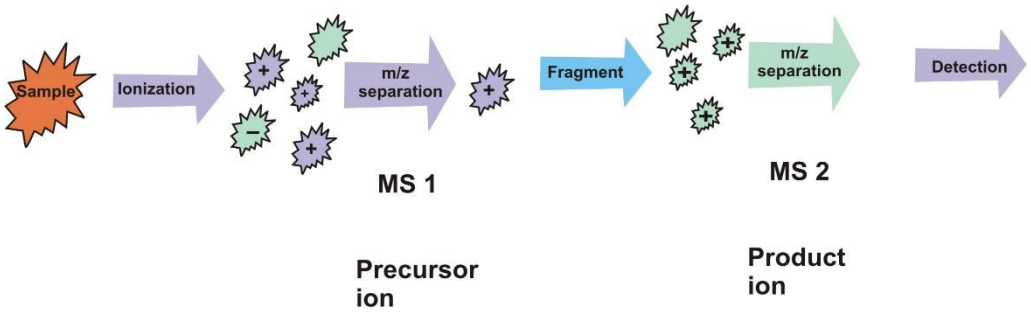


Fig. 7.9. Tandem mass spectrometry: A schematic representation

7.3.5 Applications

Tandem mass spectrometry has a large number of applications in analysing biomolecules like. They are listed below:

Analysis of protein

Tandem mass spectrometry is applied for the analysis of protein. The major information is obtained by this analysis is the amino acids sequence of protein. The entire proteins are established to a mass analyzer and this is considered as "top-down proteomics". Proteins are transformed into minor peptides and accordingly launched into the mass spectrometer, this is considered as "bottom-up proteomics".

Analysis of oligosaccharides

Oligosaccharides are moreover investigated through the application of tandem mass spectrometry an identical pattern to protein sequencing. Fragmentation typically examines on the moreover surface of the glycosidic bond, except moreover under additional energetic circumstances during the sugar ring structure in a cross-ring cleavage. Again sprawling subscripts are utilized to assign the location of the cleavage along the chain.

Analysis of oligonucleotides

Tandem mass spectrometry has been applied for the analysis of nitrogeneous base DNA and RNA sequences.

Screening of newborns

Newborn screening is the procedure of investigation regarding genetic, metabolic and hematologic diseases in newborn babies. Such application is developed in the early 1990s and applied to detect the congenital metabolic diseases that affect blood levels of organic acids in blood.

SAQ 2

1. What is the tandem mass spectrometry?
2. Who invented the tandem mass spectrometry?
3. Elaborate collisional activation (CA) or collision-induced dissociation (CID)?
4. Indicate whether the following statements are true or false:

i) J. J. Thomson is considered as a forefather of tandem mass spectrometry ( ).

ii) Tandem mass spectrometer is a combination of two mass spectrometers ( ).

iii) In tandem mass spectrometry in space, the partition elements are physically separated and distinct ( ).

iv) In the case of tandem mass spectrometry in time, the isolation is carried out with ions trapped in the same place, with multiple isolation steps taking place over time ( ).

v) Fragmentation of gas-phase ions is necessary for tandem mass spectrometry ( ).

7.4 SOLID PHASE PEPTIDE SYNTHESIS (SPPS)

In this unit up to now, we have studied about various techniques that are based on mass of a protein and went for analysing the protein nature and chemistry. This section is little different when compared to previous sections, as we will be studying about techniques used for peptide synthesis.

7.4.1 Introduction

Solid-phase peptide synthesis is a general technique for peptide synthesis. This synthesis is generally performed on a solid support. The term “solid support” is used to denote the matrix upon which chemical reactions are performed. This is a method in which molecules are covalently bound on a solid support material and synthesised step-by-step in a single reaction container using selective protecting group . This is also known as “SPPS” method.

Invention

In 1963, Mr. Bruce Merrifield developed solid phase peptide synthesis on cross-linked polystyrene beads. He awarded Nobel Prize for this work. He had to tackle three major challenges regarding to the development and acceptance of solid-phase peptide synthesis. These challenges were (1) To reduce the concept of peptide synthesis on an insoluble support (2) Overcome the resistance of synthetic chemists to this novel approach, and (3) To establish that a biochemist had the scientific credentials to effect the proposed revolutionary change in chemical synthesis.

7.4.2 Principle of SPPS

Principle of SPPS is founded on repeated cycles of deprotection-wash-coupling-wash (Fig. 7.10). Initiation of SPPS is carried out by the coupling of the C-terminus of the first amino acid to an activated solid support. This solid support is chemically unreactive polystyrol. The resin acts as the C-terminal protecting group, the immobilized protein can be engaged during a filtration procedure. The liquid-phase reagents and by-products of synthesis are flushed away. A single N-protected amino acid unit is coupled to the free N-terminal amine of a solid-phase attached peptide. This component is then deprotected, revealing a new N-terminal amine to which a further amino acid may be attached.

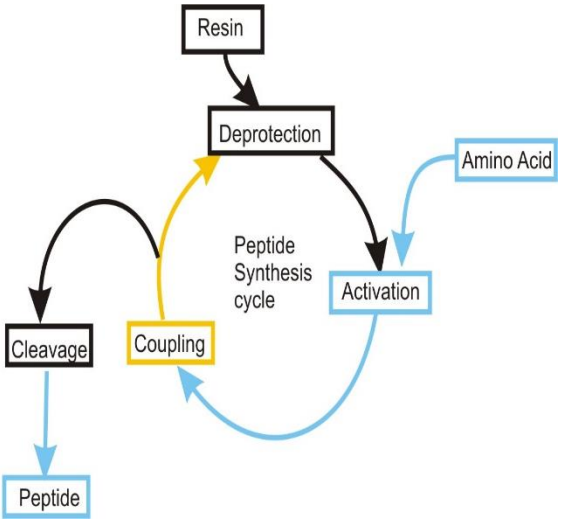


Fig. 7.10: Solid-phase peptide synthesis cycles of (1) deprotection, (2) activation of the amino acids, (3) coupling and (4) detachment of mature peptide.

7.4.3 Methodology of SPPS

Methodology of SPPS is started by the use of solid support (Fig.7.11). The solid support consists of small, polymeric resin beads. These beads contain the reactive groups (such as amine or hydroxyl groups) and linked to the nascent peptide chain. Excess reagents and side products can be eliminated by washing and filtration. The peptides are covalently attached to the solid support throughout the synthesis. Each amino acid to be coupled to the peptide chain N-terminus must be protected on its N-terminus and side chain using appropriate protecting groups such as **Boc (acid-labile)** or **Fmoc(base-labile)**, depending on the side chain and the protection strategy used.

The SPPS procedure is completed by repeated cycles of alternate N-terminal deprotection and coupling reactions. Washing of resin is performed in each step. First, the coupling of an amino acid to the resin is completed. Consequently, the amine is deprotected, and afterwards coupled with the free acidic group of the second amino acid. This cycle is repeated for many times to synthesize the desired sequence. SPPS cycles may also include capping steps which block the ends of amino acids for avoiding the reactions. After completing the synthesis, the newly synthesized peptide is cleaved from the solid support and simultaneously eliminating all protecting groups by using a reagent strong acid like trifluoroacetic acid. The newly synthesized peptide can be precipitated from a non-polar solvent like diethyl ether in order to remove organic soluble by products.

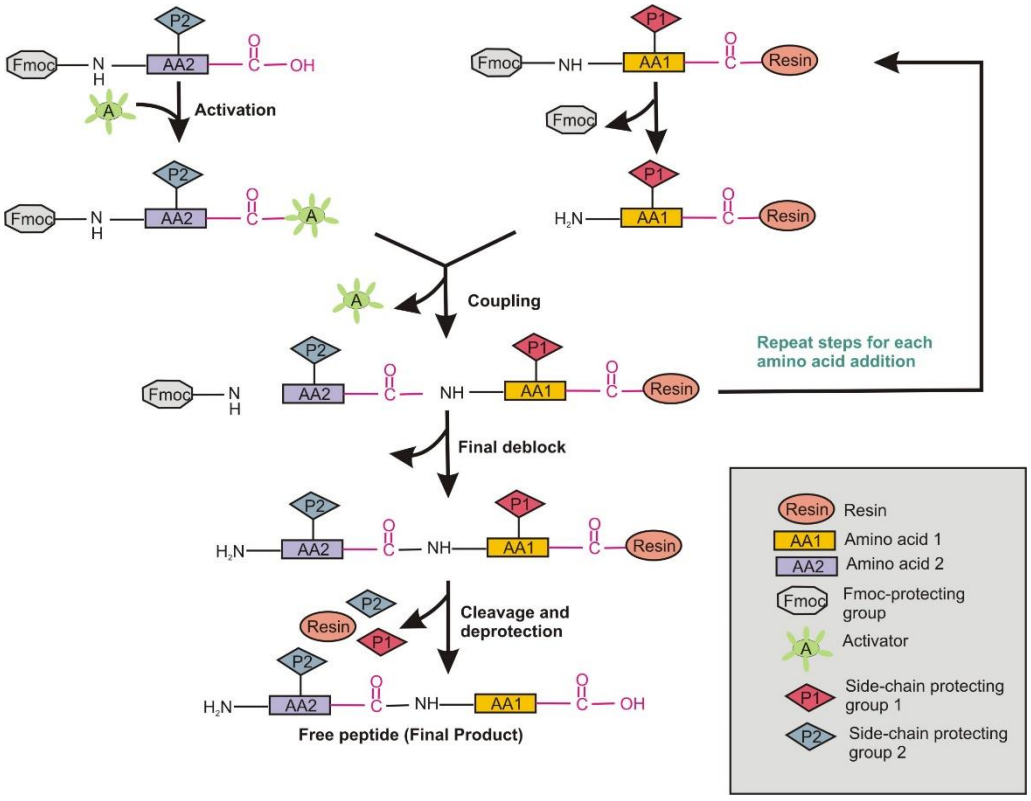


Fig.7.11: Schematic and stepwise representation of solid-phase peptide synthesis methods.

7.4.4 Applications

- Solid-phase peptide synthesis is mainly used for the synthesis of large variety of peptides.
- The small fragments of DNA, RNA, and modified oligonucleotides are also synthesised by the solid-phase method.
- The oligonucleotides can be synthesised on solid phase using a DNA/RNA synthesizer.

SAQ 3

1. Define solid-phase peptide synthesis?
2. Who invented the solid-phase peptide synthesis?
3. Enlist applications of solid-phase peptide synthesis?
4. Indicate whether the following statements are true or false:

i) Solid-phase peptide synthesis is generally occurring on a solid support( )

ii) Mr. Bruce Merrifield developed solid phase peptide synthesis on cross-linked polystyrene beads ( )

iii) Principle of SPPS is founded on repeated cycles of deprotection-wash-coupling-wash ( )

iv) The SPPS procedure is completed by repeated cycles of alternate N-terminal deprotection and coupling reactions. ( )

v) The newly synthesized peptide can be precipitated from a non-polar solvent like diethyl ether in order to remove organic soluble by products ( ).

7.5 SUMMARY

- Mass spectrometry is a technique and applied to determine the chemical composition and the structure of a molecule. This is carried out by converting the molecule into ionized state and measures their mass to charge ratio.
- The history of mass spectrometry was started from the inventive work of English scientist J.J. Thomson. He improved the work of Wilhelm Wien and developed the first canal rays-based mass spectrometry.
- Tandem mass spectrometry (MS/MS) can be revealed as one broad-spectrum approach linking at slightest two steps of mass analysis; moreover, in combination with a separation process or in a chemical reaction that establishes a variation in the mass or charge of an ion.
- J. J. Thomson is considered as a forefather of tandem mass spectrometry.
- Theory of tandem mass spectrometry can be known in two methods: in space by the coupling of two mass spectrometers, or in time by a proper series of proceedings in an ion storage apparatus.
- Tandem mass spectrometry is applied for the analysis of protein. The major information is obtained by this analysis is the amino acids sequence of proteins.
- Mr. Bruce Merrifield developed solid phase peptide synthesis on cross-linked polystyrene beads. Solid-phase peptide synthesis is a general technique for peptide synthesis.
- This synthesis is generally occurring on a solid support. The term “solid support” is used to denote the matrix upon which chemical reactions are performed.
- This is a method in which molecules are covalently bound on a solid support material and synthesised step-by-step in a single reaction container using selective protecting group . This is also known as “SPPS” method.

7.6 TERMINAL QUESTIONS

1. Explain and elaborate the principle of mass spectrometry?
2. Describe the mechanism of action of the mass spectrometry?
3. Describe the major components of the mass spectrometer?
4. Enlist applications of the mass spectrometry?

5. Explain and elaborate the principle of tandem mass spectrometry with the help of a neat diagram?

6. Write a note on applications of tandem mass spectrometry?

7. Draw a labelled flow chart explaining the principle of solid-phase peptide synthesis?

7.7 ANSWERS

SAQ 1

1. Mass spectrometry is a technique and applied to determine the chemical composition and the structure of a molecule. This is carried out by converting the molecule into ionized state and measures their mass to charge ratio.

2. J.J. Thomson invented the mass spectrometry technique.

3. Mass spectrometer needs to perform three functions: generation of ions, separation of ions and detection of ions.

4. i) magnetic field, the mass to charge ratio ii) gas phase ions iii) mass iv) electric and magnetic.

SAQ 2

1. Tandem mass spectrometry (MS/MS) can be reflected as one broad-spectrum method connecting at least two steps of mass analysis, either in conjunction with a dissociation process or in a chemical reaction that grounds an alteration in the mass or charge of an ion.

2. J. J. Thomson invented the tandem mass spectrometry.

3. Post-source fragmentation is generally used in a tandem mass spectrometry experiment. Collision-induced dissociation (CID) is also known as collisionally activated dissociation (CAD), involves the collision of an ion with a neutral atom or molecule in the gas phase and following dissociation of the ion.

4. i) True ii) True iii) True iv) True v) True.

SAQ 3

1. Solid-phase peptide synthesis is a general technique for peptide synthesis. This synthesis is generally occurring on a solid support. The term “solid support” is used to denote the matrix upon which chemical reactions are performed.
2. In 1963, Mr. Bruce Merrifield developed solid phase peptide synthesis on cross-linked polystyrene beads.

3. Solid-phase peptide synthesis is mainly used for the synthesis of large variety of peptides. The small fragments of DNA, RNA, and modified oligonucleotides are also synthesised by the solid-phase method. The oligonucleotides can be synthesised on solid phase using a DNA/RNA synthesizer.

4. i) True ii) True iii) True iv) True iv) True v) True.

Terminal Questions

Ans.1. Refer section 7.2.2.

Ans.2. Refer section 7.2.1.

Ans.3. Refer section 7.2.3.

Ans.4. Refer section 7.2.5.

Ans.5. Refer section 7.3.2.

Ans.6. Refer section 7.3.5.

Ans.7. Refer section 7.4.2.

7.8 FURTHER READINGS

1. Introduction to protein mass spectrometry. Ghosh PK. Academic Press, USA, 2015.
2. Protein Analysis using Mass Spectrometry: Accelerating Protein Biotherapeutics from Lab to Patient. Mike S. Lee, Qin C. Ji. John Wiley & Sons, -2017.
3. Protein and Peptide Analysis by Mass Spectrometry. J. R. Chapman. Humana Press, 1996.
4. Protein Mass Spectrometry. Whitelegge J. Elsevier, 2008.
5. Protein Sequencing and Identification Using Tandem Mass Spectrometry. Michael Kinter, Nicholas E. Sherman. John Wiley & Sons, -2005.