

Lecture 15: Introduction to mass spectrometry-I

Mass spectrometry (MS) is an analytical technique that measures the mass/charge ratio of charged particles in vacuum. Mass spectrometry can determine mass/charge ratio with high accuracy. Molecules in a test sample are converted to gaseous ions that are subsequently separated according to mass/charge ratio. Several types of experiments can be performed with mass spectrometry. We shall see a few examples in coming lectures.

A typical mass spectrometry instrument has three components as shown in Fig. 1.

1. Ion source
2. Analyzer
3. Detector: The detector records the current produced when an ion passes by or hits a surface. Several types of detector are used like electron multiplier, Faraday cups and ion to photon detectors.

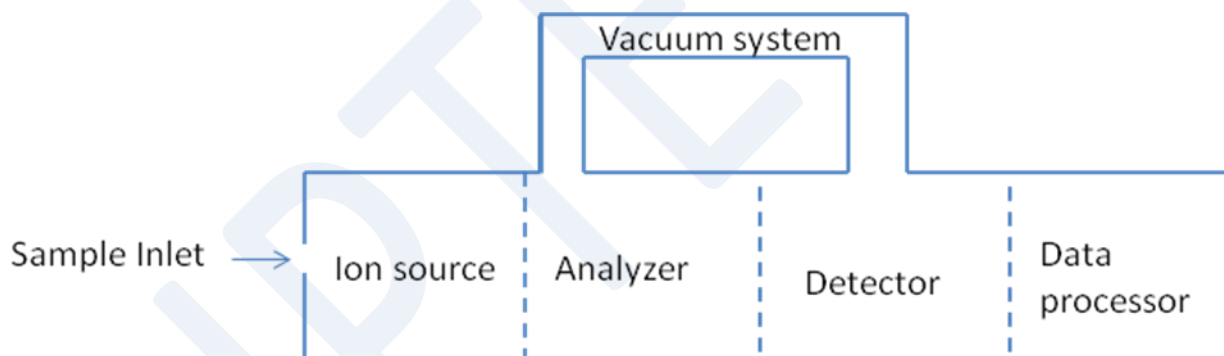
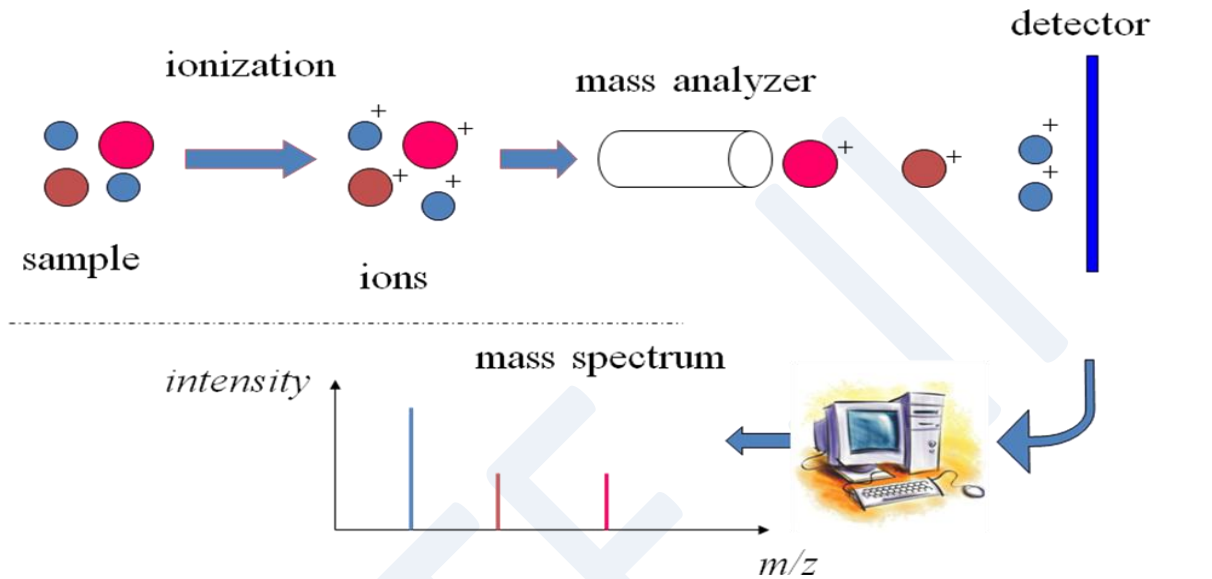


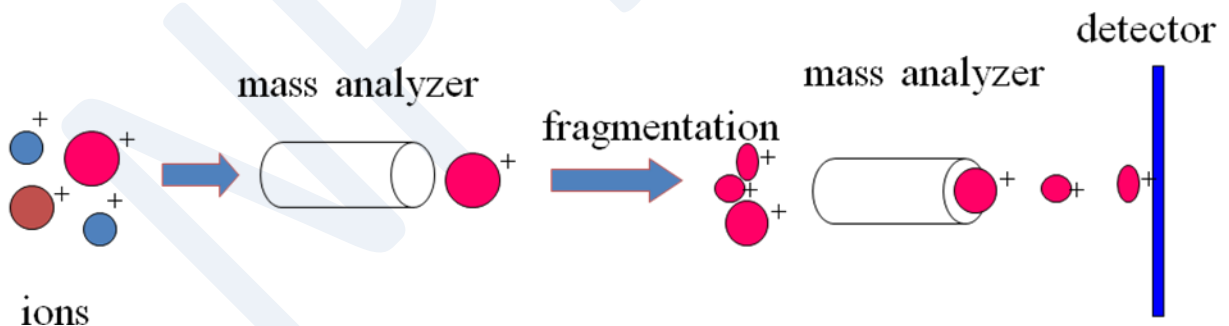
Figure 1: Basic components of mass spectrometry

All mass spectrometry operate under vacuum (10^{-6} torr pressure). Without high vacuum, the ions produced in the source will not reach the detector. At atmospheric pressure, the mean free path of a typical ion is around 52 nm; at 1mtorr, it is 40 mm; and at 10^{-6} torr, it is 40 m. Sample inlet may be coupled to a liquid chromatography system (call LC-MS), gas chromatography system (GC-MS) or capillary electrophoresis system.

We shall study components of mass spectrometry (MS) in detail. A simplified scheme of mass spectrometry (MS) and MS-MS (tandem mass spectrometry) is shown in Fig 2)



A simplified scheme of mass spectrometry (MS)



A simplified scheme of MS-MS

Figure 2: A simplified scheme of mass spectrometry and Tandem mass spectrometry (also called MS MS). In MS-MS, after first analyzer, analytes are fragmented and fragments are analyzed in second analyzer.

Ion source:

There are several types of ionization methods in mass spectrometry. The physical basis of ionization methods are very complex and outside the scope of the course. Most common methods are:

(a) Matrix-assisted laser desorption/ionization (MALDI)

This method of ionization is a soft ionization method and results in minimum fragmentation of sample. This method is used for non-volatile, and thermally labile compounds such as proteins, oligonucleotides, synthetic polymers. Sample is mixed with 1000 times molar excess of matrix and spotted onto a metal plate and dried. Matrix plays a key role in this technique by absorbing the laser light energy and causing a small part of the target substrate to vaporize. Although, the process of forming analyte ions is unclear, it is believed that matrix which has labile protons, such as carboxylic acids, protonates neutral analyte molecules after absorbing laser light energy. Scheme of MALDI is shown in Fig. 3 and some common matrices are listed in Table 1

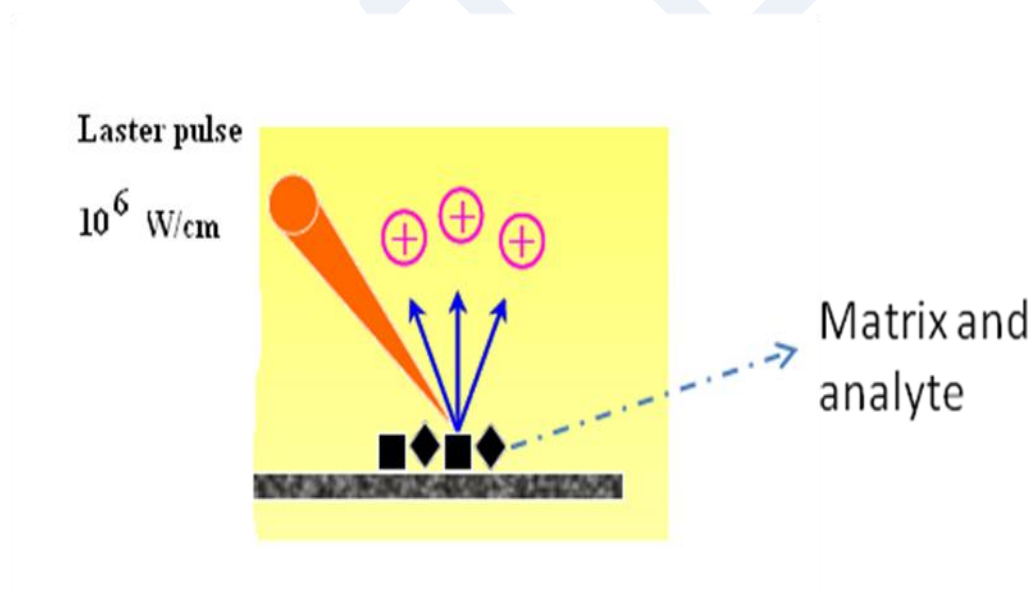
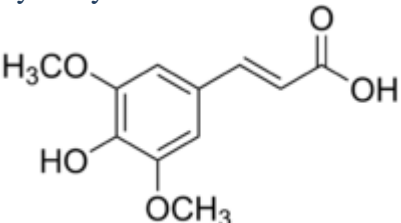
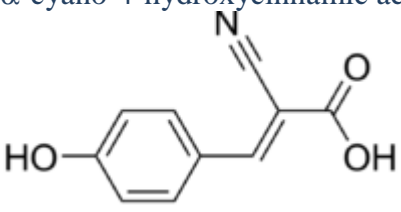


Figure 3: Scheme of Matrix-assisted laser desorption/ionization (MALDI)

Table 1: Few common matrices used in MALDI

Matrix	Application
3,5-Dimethoxy-4-hydroxycinnamic acid 	Higher mass biopolymer
α -cyano-4-hydroxycinnamic acid 	Protein, peptides, organic compounds
2,5 dihydroxy benzoic acid	Oligonucleotide
Trihydroxyacetophenone	Peptides, Oligonucleotide

(b) Electrospray Ionisation (ESI)

Electrospray Ionisation (ESI) is a preferred method of ionization when the sample is in liquid form. This is also a soft method of ionization and results in less fragmentation. ESI is a very valuable method for analysis of biological samples. The method was developed by John Fenn and he shared 2002 Nobel prize in chemistry for this work. The analyte is introduced either from a syringe pump or as the eluent flow from liquid chromatography with a flow rate $1\mu\text{l min}^{-1}$. The analyte solution passes through the electrospray needle (Stainless steel capillary with 75-150 μm internal diameters) that has a high potential difference (with respect to the counter electrode) applied to it (typically in the range from 2.5 to 4 kV). This forces the spraying of charged droplets from the needle with a surface charge of the same polarity to the charge on the needle. As droplet moves towards counter electrode cone (which passes it to

analyzer), solvent evaporation occurs and droplet shrinks until it reaches the point that the surface tension can no longer sustain the charge (the Rayleigh limit) and at that point droplets break. This produces smaller droplets and the process is repeated. Finally after all solvent evaporated, charge is passed on to analyte. These charged analyte molecules can have single or multiple charges (Fig. 4)

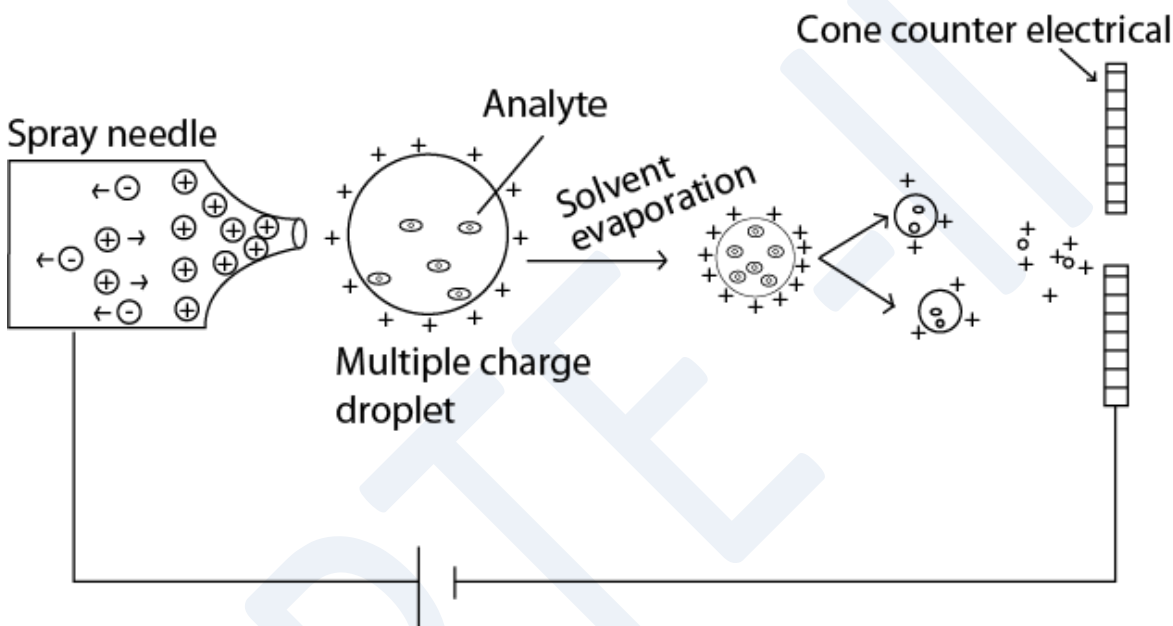


Figure 4: A schematic of the mechanism of ion formation in ESI.

(c) *Electron ionization*

Electron Ionization (EI) works well for many gas phase molecules, but it results in extensive fragmentation and molecular ions are not observed for many compounds. Fragmentation mass spectra are sometime useful because it provides structural information of a molecule.

The electron beam is produced by a filament of rhenium or tungsten wire by thermionic emission. When cathode filament of rhenium or tungsten is heated at temperature over 1000 K, electrons are emitted. The generated electrons are accelerated to 70 eV which results in

electron beam. The volatile sample or sample in gaseous phase containing neutral molecules is introduced to the ion source in a perpendicular direction to the electron beam. Electron impact on the analyte results in either loss of electron (to produce cation) or gain of electron (to produce anion). Chemical bonds in organic molecules are formed by pairing of electrons. Electron impact may knock out one of the electron. This leaves the bond with a single unpaired electron. This is radical as well as being cation written as M^+ , where (+) indicates ionic state while (·) indicates radical. Electron impact may result in electron capture (extra unpaired electron). This generates a radical as well as being anion written as M^- , where (-) indicates ionic state while (·) indicates radical (Fig. 5)

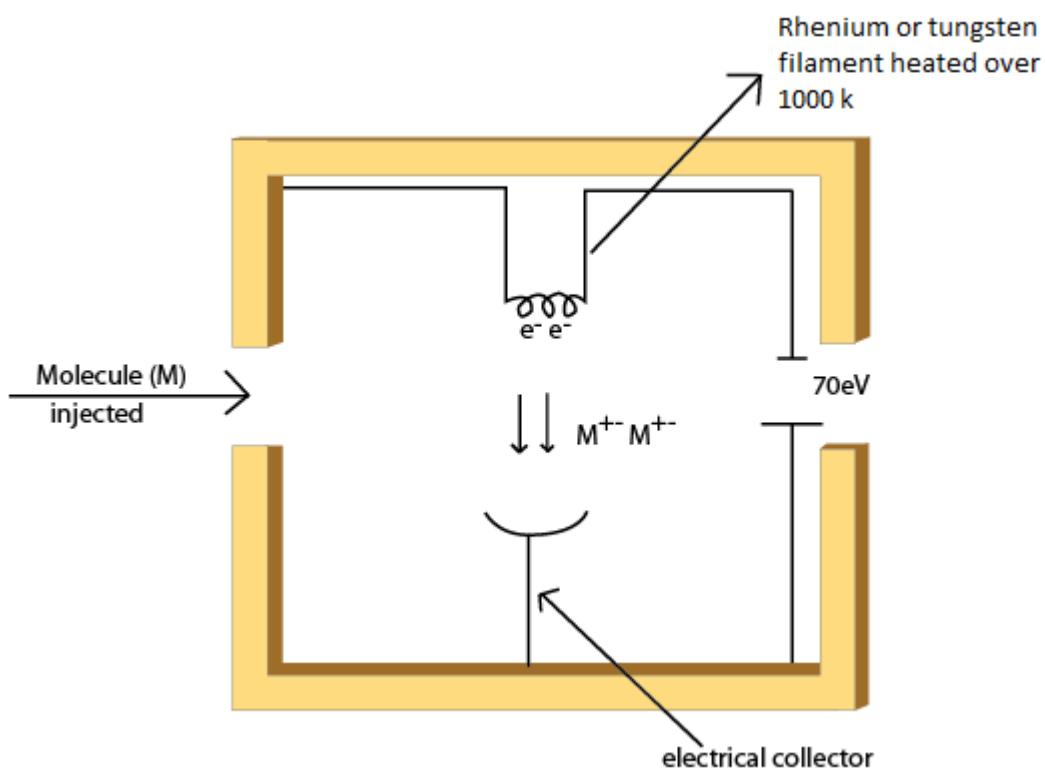
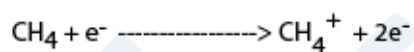


Figure 5: A schematic of the mechanism of ion formation in electron ionization

(a) Chemical Ionization

Set-up for chemical Ionization is similar to electron impact ionization. However, in this method, a reagent gas like CH₄ is injected in the ion chamber. Due to electron impact, the reagent gas in the chemical ionization source gets ionized. This follows injection of analyte molecule. Analyte molecules undergo many collisions with the reagent gas. The reagent gas ions in this cloud react and produce adduct ions as shown in figure, which are excellent proton donors for analyte.

Electron impact on reagent gas



Reaction of reagent gas to form 1 on



Reaction of Reagent gas 1 on with analyte

