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State of the Art of Hard and Soft Ionization Mass Spectrometry

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Abstract

The principles of hard and soft ionization sources, providing some details on the practical aspects of their uses as well as ionization mechanisms are discussed. The conditions and uses of hard ionization methods such as electron impact, thermal ionization and inductively coupled plasma techniques are discussed. Moreover, new generation of soft ionization methods such as matrix-assisted laser desorption/ionization, electro spray ionization and direct analysis in real time are illustrated.

Introduction

Mass spectrometers determine the masses of the molecules by measuring their mass-to-charge ratio (m/z) of its ion. Ions are generated by inducing either the loss or gain of a charge from a neutral species. Once ions are directed into a mass analyzer where they are separated and detected according to their m/z. The result of ionization, ion separation, and ion detection is a spectrum that can provide molecular mass and even structural information[1].

For small and elemental species the ionization is carried by hard ionization methods such as electron impact, thermal or surface ionization and plasma conditions. In recent years, the efforts of many investigators have led to new soft ionization techniques for producing ions of too large species.

Electron Impact (EI)[2] method was the primary ionization source for mass analysis. However, EI limited chemists and biochemists to small molecules below the mass range of common bio-organic compounds. This limitation motivated scientists such as Fenn et al[3&4], Tanaka[5], Karas[6], among others to develop the new generation of ionization techniques, including Matrix-Assisted Laser Desorption/ionization (MALDI), and Electro Spray Ionization (ESI) methods. These techniques have revolutionized biomolecular analyses, especially for large molecules. The most recent soft ionization technique is the Direct Analysis in Real Time (DART) method [8&9] .

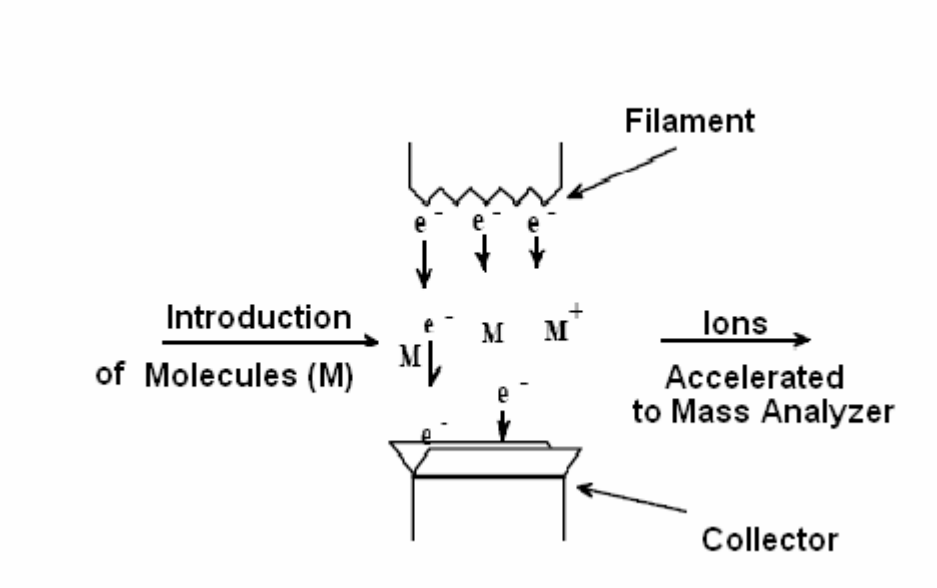
In the following the principles of ionization sources, providing some details on the practical aspects of their use as well as ionization mechanisms are discussed.

Electron Impact Technique

EI ionization technique is one of the most important ionization sources for the routine analysis of small, thermally stable molecules. EI generates many fragment ions; therefore it is defined as a "hard" ionization source. The fragmentation information can also be very useful. By employing databases containing electron ionization mass spectra, it is possible to identify an unknown molecular structure.

The sample must be introduced as a gas by either boiling the sample, or by introduction a gas through a capillary. The capillary is often the output of a capillary column from a gas chromatography instrument. The capillary column provides separation which is known as gas chromatography mass spectrometry (GC/MS). El excites the molecule, thus causing electron ejection ionization and fragmentation. The utility of electron ionization decreases significantly for

compounds above a molecular weight of 400 mass units because the required thermal desorption of the sample often leads to thermal decomposition before vaporization is able to occur.



Electrons ejected from a heated filament are accelerated through an electric field at 70 V to form a continuous electron beam. The sample molecule is passed through the electron beam. Electrons, containing kinetic energy (70 eV) transfer some of their energy to the molecule. This transfer results in ionization (electron ejection) with the ion which usually has about 5 eV excess energy.

$$M + e\text{-}\ (70\ eV) \rightarrow M^{+}\ (\sim 5\ eV) + 2e\text{-}\ (\sim 65\ eV)$$

Excess internal energy (5 eV) in the molecule leads to some degrees of fragmentation.

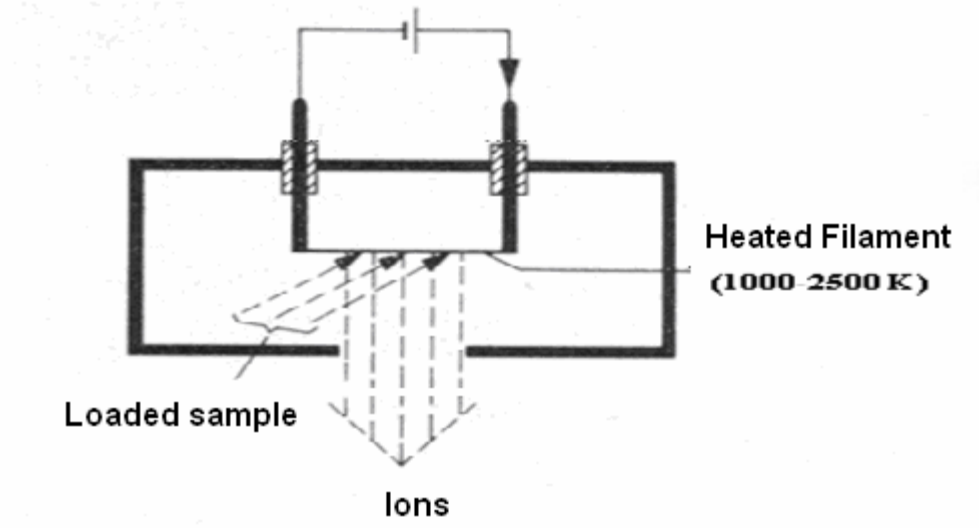
$$M^{+} \rightarrow \text{molecular ions} + \text{fragment ions} + \text{neutral fragments}$$

Thermal Ionization Technique

In Thermal Ionization Mass Spectrometry (TIMS) the ions are coming out of the filament and are directed into a mass spectrometer to analyze the present elements or isotopes in the sample[(1)]. In thermal or surface ionization method, material is loaded onto a filament which is heated and some of the loaded element could be ionized. The ionization probability is a function of the filament temperature, the work-function of the filament substrate and the ionization energy of the element. This is expressed by the Saha equation:

$$(i/n) = (g_1/g_0) \exp [(WF-I)/kT]$$

i/n = ion to neutral ratio , g_1, g_0 = statistical weights of ion and neutral states ,WF = surface work function , I = element Ionization Energy , k = Boltzmann's constant
and T = surface temperature

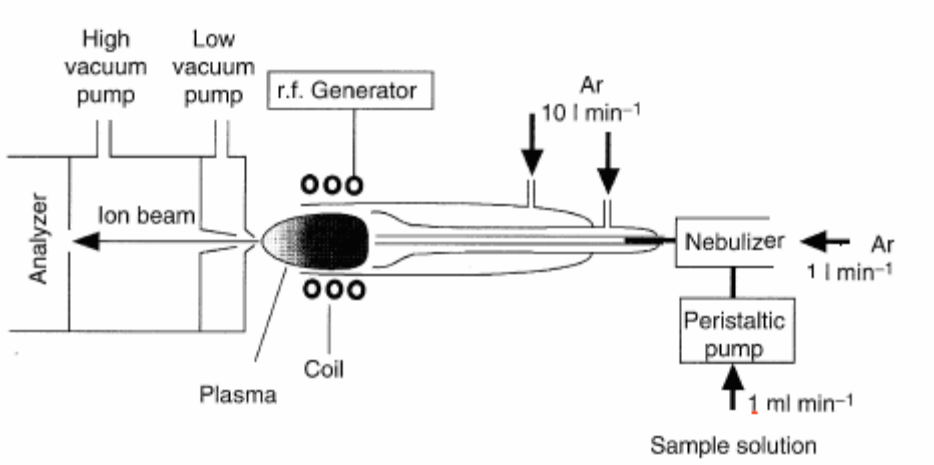


This technique is one of the most important techniques for isotopic ratio methods especially for heavy elements and age dating methods.

Inductively Coupled Plasma Mass Spectrometry Method

Inductively Coupled Plasma (ICP) is a high temperature plasma of partly ionized argon which is sustained with a radiofrequency electric current, that acts to produce ions[7]. The electric current is transferred to the plasma by an induction coil, wrapped around a configuration of concentric quartz tubes namely, the plasma torch. The common operating frequencies are 27.12 and 40.68 MHz and operating power is in the range between 800 and 1500 W. The plasma is sustained within a constant flow of argon gas, open to atmosphere and reaches temperatures as high as 10,000 K in the hottest part. To prevent melting of the torch a high flow rate of argon is used in the outermost tubing. The total gas consumption of a typical analytical ICP is in the range of 14 - 18 L/min. Ions from the plasma are extracted through a series of cones into a mass spectrometer.

A sample is injected into the instrument, normally by an auto sampler. The sample is atomized and delivered through a glass tube by an argon carrier gas. The sample is then exposed to radio frequency which converts the gas into the plasma. A fraction of the ions passes through a ~1mm hole (sampler cone) and then a ~0.4mm hole (skimmer cone).



ICPMS

Ionization occurs via argon plasma where argon has the advantage of being abundant and is available more cheaply than the other inert gasses. Argon has the advantage of having higher first ionization energy than all other elements except He , F and Ne.

The radio frequency causes the ionization of argon and the elements:

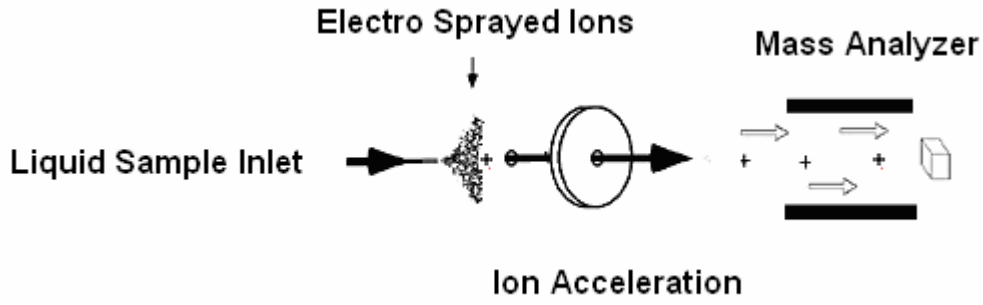
$Ar \rightarrow Ar^{+} + e^{-}$ & $M \rightarrow M^{+} + e^{-}$.

ICP-MS is a multielement technique which allows the determination of elements with concentrations down to part per trillion. Some masses are prohibited such as 40 due to the abundance of argon in the sample.

There is an increasing trend of using ICP- MS as a tool in speciation analysis, which needs a chromatograph separation.

Electro spray Ionization Technique

Electro Spray Ionization (ESI) is a method of detection of biological macromolecules. It produces gaseous ionized molecules directly from a liquid solution and operates by creating a fine spray of highly charged droplets in the presence of an electric field as illustrated in the following:

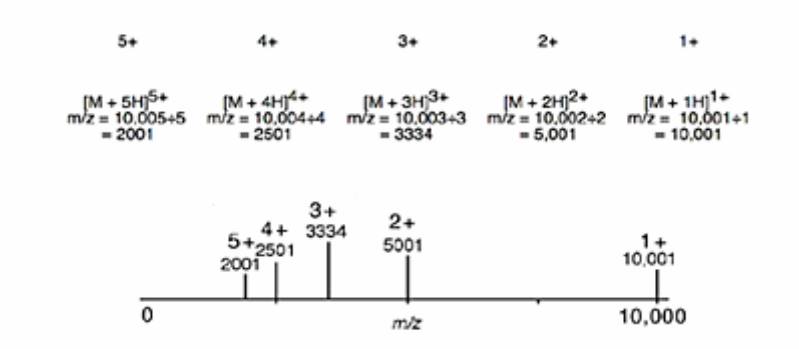


The sample solution is sprayed from a region of a strong electric field at the tip of a metal nozzle maintained at a potential in the range from 700 V to 5000 V. The nozzle serves to disperse the solution into a fine spray of charged droplets. Heat is applied to the droplets at atmospheric pressure thus causing the solvent to evaporate from each droplet. As the size of the charged droplet decreases, the charge density on its surface increases. The mutual Columbic repulsion

between like charges on this surface becomes so great that it exceeds the forces of surface tension, and ions are ejected from the droplet through a cone. Another possibility is that the droplet explodes releasing the ions. In either case, the emerging ions are directed into an orifice through electrostatic lenses leading to the vacuum of the mass analyzer.

ESl is producing multiply charged species of larger molecules. This is an important phenomenon because the mass spectrometer measures the mass-to-charge ratio (m/z) and therefore multiple charging makes it possible to observe very large molecules with an instrument having a relatively small mass range. Fortunately, the software available with electro spray mass spectrometers facilitates the molecular weight calculations necessary to determine the actual mass of the multiply-charged species.

Protein ionization is usually the result of protonation, which not only adds charge but also increases the mass of the protein by the number of added protons, which is illustrated in the following figure for biological molecule of m/z=10000 .



Many solvents can be used in ESI and are chosen based on the solubility of the compound of interest, the volatility of the solvent and the solvent's ability to donate a proton. Primary solvents such as methanol, 50/50 methanol/water, or 50/50 acetonitrile/H2O are used, while co solvents, such as 10% DMSO in water, as well as isopropyl alcohol are used to improve solubility for some compounds.

This technique has many advantages such as: practical mass range of up to 70,000 mass units, good sensitivity with femto mole to low pico mole sensitivity , typical
softest ionization method, capable of generating no covalent complexes in the gas phase ,easily adaptable to liquid chromatography , easily adaptable to tandem mass analyzers such as ion traps and triple Quadrupole instruments ,multiple charging allows for analysis of high mass ions with a relatively low m/z range instrument and no matrix interference . While it has some disadvantages such as: the presence of salts can reduce sensitivity , complex mixtures can reduce sensitivity , simultaneous mixture analysis can be poor ,multiple charging can be confusing especially in mixture analysis.

Matrix-Assisted Laser Desorption/Ionization Technique

Matrix-Assisted laser desorption/ionization (MALDI) mass spectrometry was first introduced in 1988 by Tanaka, Karas, and Hillenkamp[5&6]. It is a widespread analytical tool for peptides,

proteins, and other biomolecules .The efficient energy transfer during a matrix-assisted laser-induced desorption event provides high ion yields of the intact analyte, and allows for the measurement of compounds with sub-picomole sensitivity. In addition, the utility of MALDI for the analysis of heterogeneous samples makes it very attractive for the mass analysis of complex biological samples.

While the exact desorption/ionization mechanism for MALDI is not known, it is generally believed that MALDI causes the ionization and transfer of a sample from the condensed phase to the gas phase via laser excitation and ablation of the sample matrix. It is generally expected that ionization occurs through proton transfer or cationization during the desorption process.

A nonvolatile solid material facilitates the desorption and ionization process by absorbing the laser radiation. As a result, both the matrix and any sample embedded in the matrix are vaporized. The matrix also serves to minimize sample damage from laser radiation by absorbing most of the incident energy.

Once in the gas phase, the desorbed charged molecules are then directed electrostatically from the MALDI ionization source into the mass analyzer. Time-of-flight (TOF) mass analyzers are often used to separate the ions according to their mass-to-charge ratio (m/z).

The utility of MALDI for biomolecule analyses lies in its ability to provide molecular weight information on intact molecules especially for protein identification and characterization.

This technique has some advantages such as:
Practical mass range of up to 300,000 mass units ,typical sensitivity on the order of low femto mole to low pico mole ;soft ionization with little or no fragmentation; tolerance of salts in milli molar concentrations; and suitable for the analysis of complex mixtures.

It has some disadvantages such as: matrix background, which can be a problem for compounds below a mass of 700 mass units, possibility of photo-degradation by laser desorption/ionization and the acidic matrix which is used in MALDI may cause degradation on some compounds.

Direct Analysis in Real Time Technique

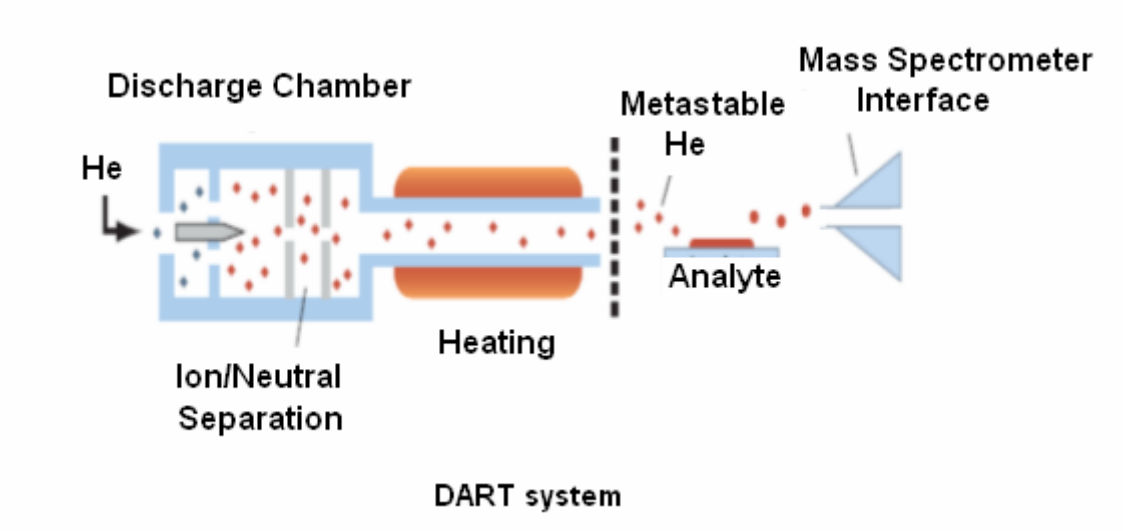
Direct Analysis in Real Time (DART) refers to an atmospheric-pressure ion source for mass spectrometry that permits analysis of gases, liquids, solids, or materials on surfaces in open air at ground potential under ambient conditions. Samples such as pills, clothing, human skin, plant material, liquid droplets, etc. are held in front of the DART source resulting in immediate formation of ions that are directed into a mass spectrometer for rapid analysis[8&9]. DART was publicly announced concurrent with the introduction of a commercial product in 2005. Samples exposed to the DART gas stream can generate ions that are carried by the gas stream into the sampling orifice of the mass spectrometer interface. The DART source operates by exposing the sample to a dry gas stream of helium or nitrogen that contains long-lived electronically excited neutral atoms or molecules ,namely "metastables". Excited states are typically formed in the DART source by creating a glow discharge in a chamber through which the gas flows.

The excited-state species can interact and ionize the sample. This process is referred to as Penning ionization, a reaction between an excited-state neutral atom or molecule M* and a substrate x that has an ionization energy with a lower energy than the internal energy of the excited-state species, resulting in the formation of a substrate radical molecular cation $x^{+\cdot}$ and an electron e-:

$$M^* + x \rightarrow x^{+\cdot} + e^-$$

The helium metastable state has an internal energy of 19.8 electron volts, which is sufficient to ionize most organic molecules.
Alternatively, the excited-state species can interact with atmospheric gases such as water to form reagent ions that undergo chemical ionization reactions that result in protonated molecules of the analyte.

$$He^* + H2O \rightarrow H2O^{+\bullet} + He^* + e^-$$
$$H2O^{+\bullet} + H2O \rightarrow H3O^+ + OH^\bullet$$
$$H3O^+ + M \rightarrow MH^+$$



DART system

DART produces relatively simple mass spectra, dominated by protonated molecules MH^+. Depending on the nature of the molecule, other species may be formed, such as $M^{+\cdot}$ from poly aromatic hydrocarbons. Fragmentation may occasionally be observed for some molecules. Multiple-charge ions and alkali metal cation adducts are never observed, but addition of ammonia or other "dopants" to the DART gas stream can be used to form single-charge adducts such as $[M+NH4]^+$.

DART is useful for small-molecule analysis. It is not a technique for analysis of large biomolecules such as proteins, although industrial polymers and some large molecules such as cellulose can be fragmented to produce characteristic "fingerprint" patterns. DART's relative insensitivity to contamination and lack of carryover between samples makes it possible to analyze many materials with little or no sample preparation. It should be noted that the major limitation for direct analysis is the ability to distinguish the sample from other environmental compounds. Most DART applications to date have been measured with a time-of-flight mass spectrometer.

DART has found a variety of applications including forensics and security, reaction monitoring and characterization of synthetic products, analysis of foods and beverages, environmental analysis, and art conservation.

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