CHEM*3440

Chemical Instrumentation

Topic 12

Mass Spectrometry

Fundamental Scheme

In general there are four steps associated with a mass spectroscopic experiment:

❶ generate gas-phase molecules from analyte (solid, liquid, solution, etc.)

❷ ionize those molecules

❸ separate the ions based on mass

❹ detect the ion beam

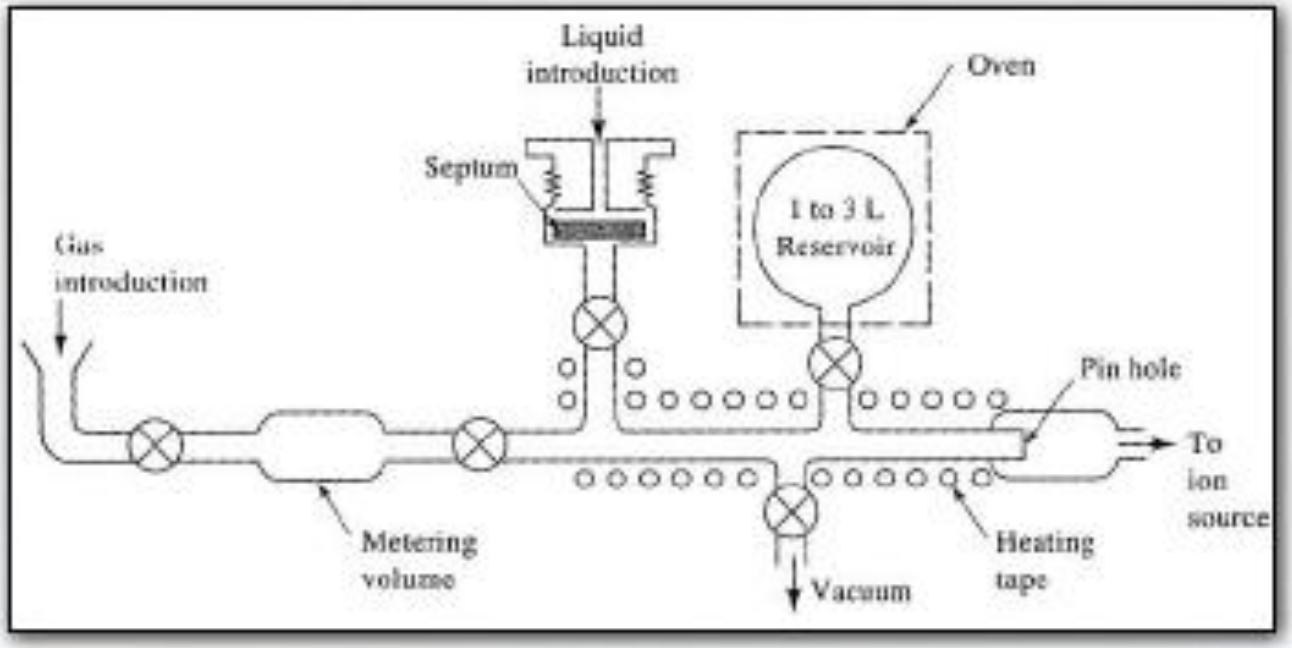
Numerous experiments are distinguished from one another by the way they handle stage 1 (LC-MS, GC-MS, SIMS, MALDI-TOF, etc.)

Different mass spec instruments are distinguished from each other by the way the accomplish both stage 2 and stage 3.

Fewer options for stage 4 exist and this is common to most instruments.

Source - Sample Injection

The mass spectrometer needs gaseous ions to analyze.
No problem if sample is already gaseous or if experiment occurring in vacuum chamber.
Heated injection port most common way of dealing with liquids and solutions. Normal boiling point < 500 °C. Maximum port temperature ~ 350 °C. Pressure ~ 10^{-6} Torr.



Source - Ionization

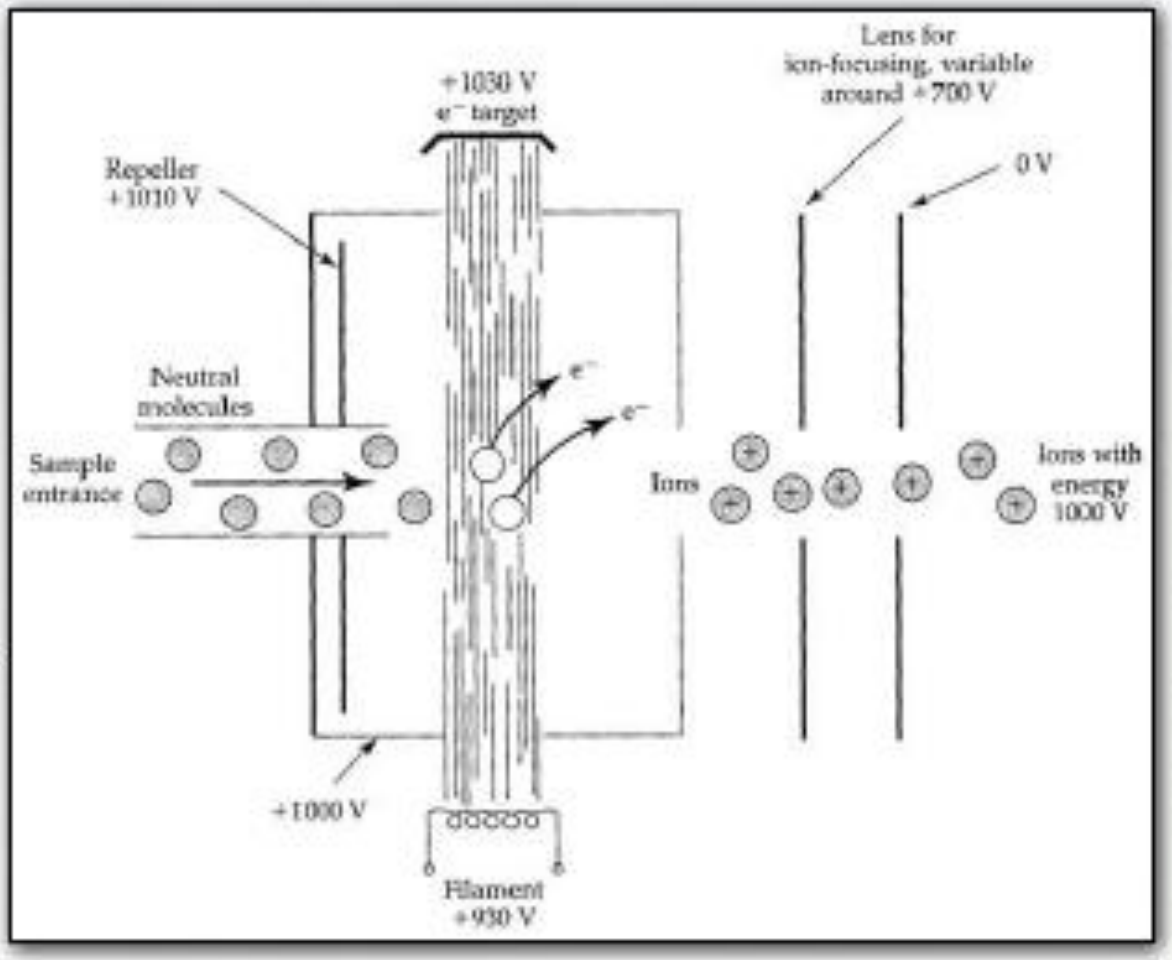
Once we have produced gas phase molecules, we can start to look at the ionization process.

With some techniques, ionization occurs in conjunction with the vapourization step (we'll discuss them later). If it does not, then we need to create the ions.

- Electron Impact ionization (EI)
- Chemical ionization (CI)
- Thermal ionization (TI)

Electron Impact Ionization

Neutral gas phase molecules drift into ionization region. Energetic electron beam (50 - 100 eV) intersects molecules. Collisions create cations. Field extracts ions from ionizer and focuses and accelerates (500 - 1000 eV) ion beam towards mass analyzer.



EI Fragmentation

It only takes about 5 to 15 eV of energy to ionize the molecule. The rest of the electron energy is available to induce molecular bond rupture.

Fragmentation with ET is extensive.

This is useful since by studying the fragmentation pattern, one can discern the likely structural components of the original molecule and develop a model for its structure.

Fragmentation with EI is so extensive, however, that the parent ion – the ion arising from the original molecule – is often not detectable. EI really "blows apart" the target molecules.

Chemical Ionization

Chemical Ionization (CI) is a means of "softening" the fragmentation effect of EI. A buffer gas, often methane CH_4, is added in a large excess (~x100 fold) over the analyte. The electron beam ionizes the methane, gas phase reactions produce molecular ions such as CH_5^+ which collide with analyte molecules (M) to gently produce the analyte ions (MH^+).

Can perform CI in same unit with EI except for much higher pressure.

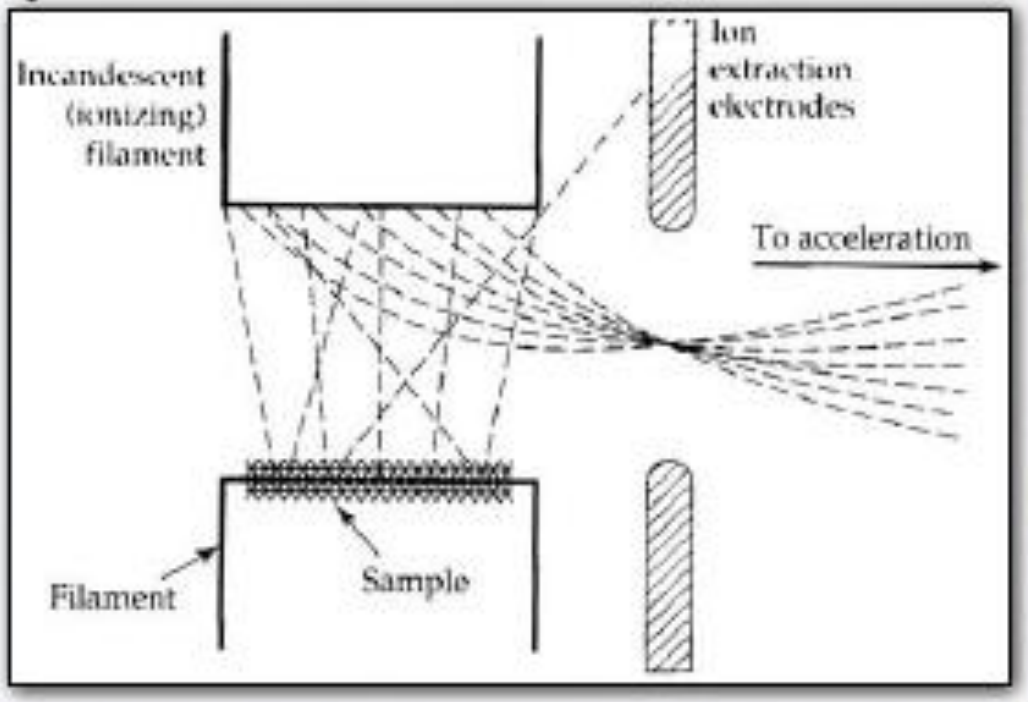
Must provide additional pumping speed to move extra buffer gas out of ionizer so as to not degrade vacuum in analyzer.

Ionization process much gentler and it tends to produce ions with an extra proton mass.

Thermal Ionization

Was original ionization method. Now used very rarely, except in isotope ratio studies in nuclear and geochemistry.
Coat one filament with material to be ionized; let it dry and harden.
Introduce this filament into the vacuum chamber, next to (~mm) another filament.
Heat first filament to desorb analyte. Heat second filament even hotter.

Desorbing molecules are ionized by thermal contact with hotter filament.
Ions extracted, accelerated, and focused into mass analyzer as before. Has been source of choice for quantitative work.



Field Ionization

When a large potential difference is applied across an object fashioned to come to an atomically sharp point, the electric field in this region is HUGE (10^8 V/cm). These fields, across a single molecule, have enough potential to pull an electron directly from the molecule.

Electron tunnels from molecule to tip, imparting little energy into vibrational or rotational degrees of freedom. Very little fragmentation occurs.
Problem is much lower sensitivity than for EI.

An SEM of a carbon microneedle

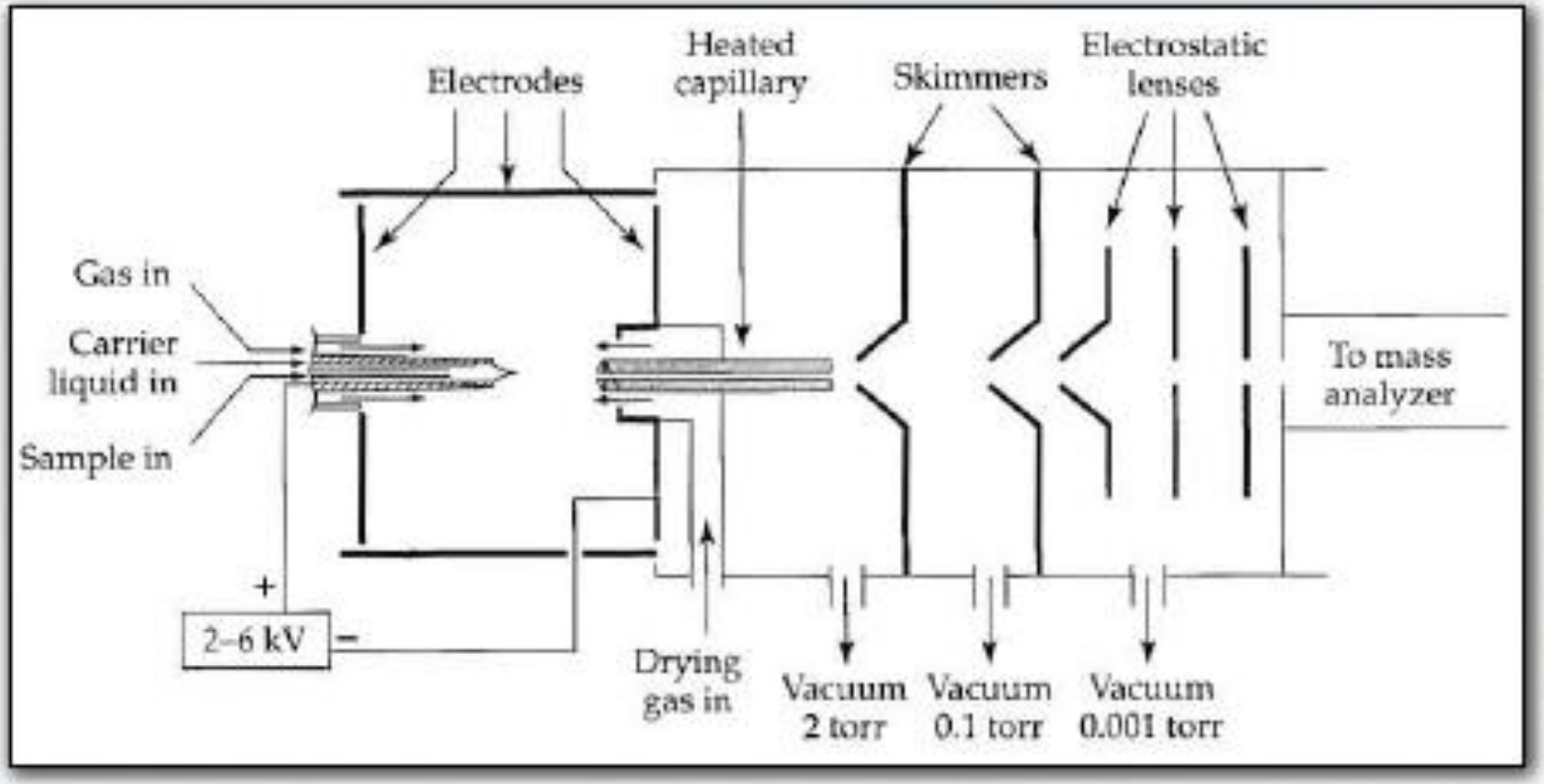
Source - Vapourization/Ionization

In recent years, many new techniques for vapourizing and ionizing samples have been developed in an effort to be more gentle and to access much larger molecules, driven , of course, by a need to study biomolecular systems. These are

- Electrospray Ionization (ESI)
- Field Desorption
- Inductively Coupled Plasma (ICP)
- Laser Ablation
- Matrix Assisted Laser Desorption Ionization (MALDI)
- Fast Atom Bombardment (FAB)
- Ion Sputtering

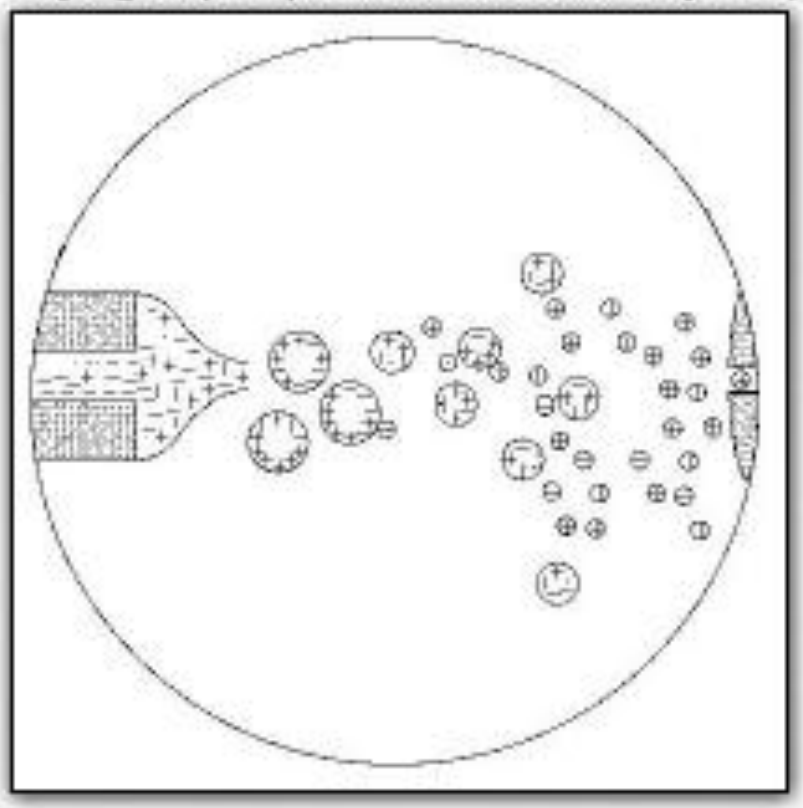
Electrospray Ionization

The most gentle ionization technique. Produces multiply charged ions.
Based on Ion Evapouration process.



Ion Evapouration Process

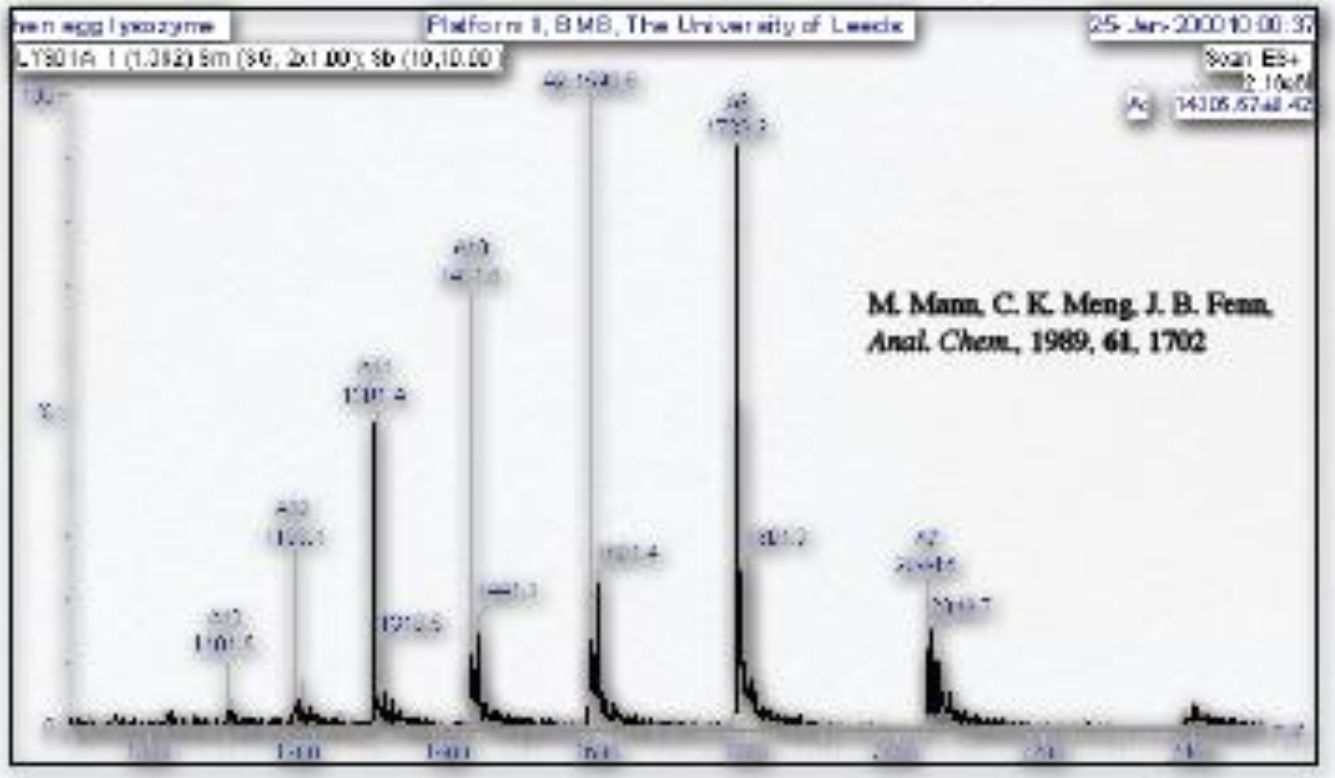
Solvent with analyte picks up ionic charge in nozzle. Spray produces μm size droplets. They flow towards the capillary against a counter flow of drying air; droplets shrink in size by evapouration. At a certain point,



the surface ionic charge repulsion exceeds the surface tension and the droplet breaks into many smaller droplets. This process repeats itself many times. Final stage produces desolvated, multiply charged analyte ions which move down capillary into mass analyzer.

Multiple Charging in ESI

Multiple H^+ ions can be attached in this process. Each cluster of peaks is the same protein but with an additional proton. The ions showing up at 1431.6 m/z is the same as the ion at 1301.4 m/z except with 1 less H^+.



Multiple Charging con't

Let MW be the molecular weight of the unknown ion. There are n H^+ ions attached to it at 1431.6 and so there are n+1 H^+ ions attached to it at 1301.4.

$$1431.6 = \frac{MW(\text{protein}) + n_{1431.6} MW(H^+)}{n_{1431.6}} \qquad 1301.4 = \frac{MW(\text{protein}) + n_{1301.4} MW(H^+)}{n_{1301.4}}$$

but $n_{1301.4} = n_{1431.6} + 1 = n + 1$

Rewrite and combine first two equations

$$n1431.6 - nH^+ = MW(\text{protein}) = (n+1)1301.4 - (n+1)H^+$$

Solve for n

$$n1431.6 = (n+1)1301.4 - (n+1)H^+ + nH^+ = (n+1)1301.4 - H^+$$

$$n(1431.6 - 1301.4) = 1301.4 - H^+ = 1301.4 - 1.008 = 1300.4$$

$$n = \frac{1300.4}{1431.6 - 1301.4} = \frac{1300.4}{130.2} \approx 10$$

Multiple Charging con't

With this value of n, we can go back to either of the original expressions to find the MW of the unknown protein.

$1431.6 = (MW + 10(H^+))/10$

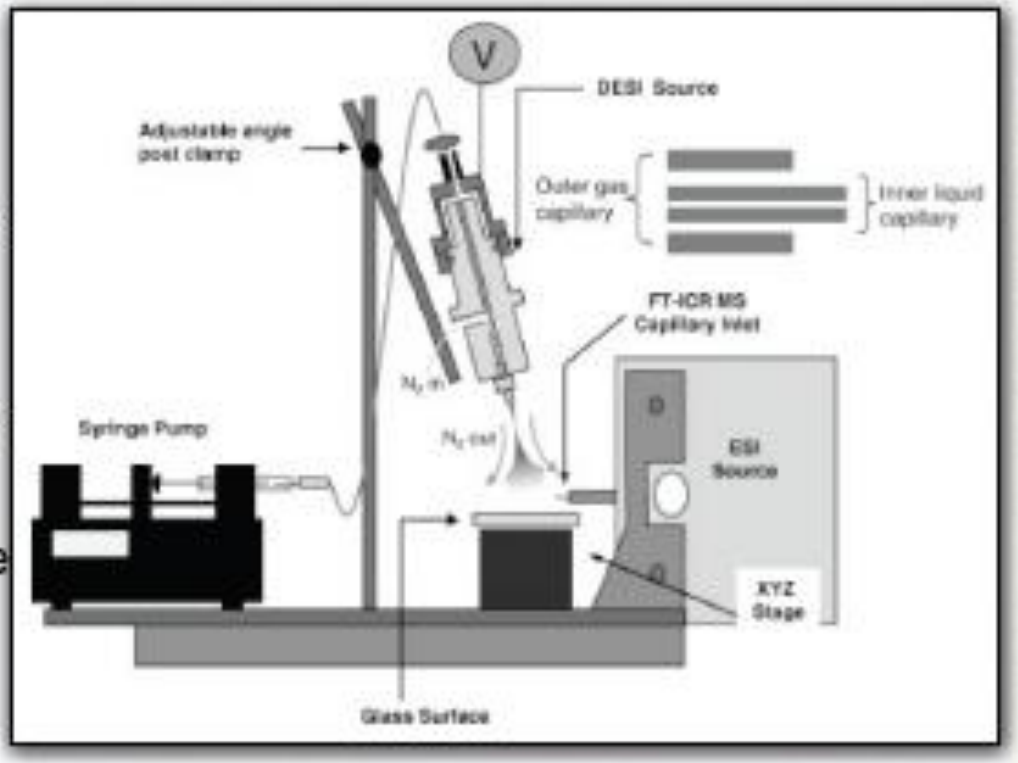
$10(1431.6)-10(1.008) = 14305.9$ Da

The mass of hen's egg lysozyme is 14305.9 Da. A mass of 1305.14 would be predicted from known average atomic masses. With ESI was can expect a mass precision of 0.01%, which would be 1.4 Da in this case. The result is well within the acceptable error limits.

In the past, a small molecule's mass was often reported as amu (atomic mass units), but larger molecule's mass seemed to like to be given in Da (daltons). It is the same thing; 1/12 the mass of a ^{12}C atom. The more modern convention is to use Da for mass of atoms and molecules. But I'm sure amu will die with difficulty for atoms and small molecules.

Desorption ESI (DESI)

A variation of the ESI ionizer uses a high pressure stream of N_2 to nebulize a solvent, forming a high velocity jet of charged solvent droplets. These are directed at a surface upon which the analyte has been placed. When these droplets strike the surface, they pick up some analyte and bounce back into the gas phase where they enter the mass spec through a small capillary.



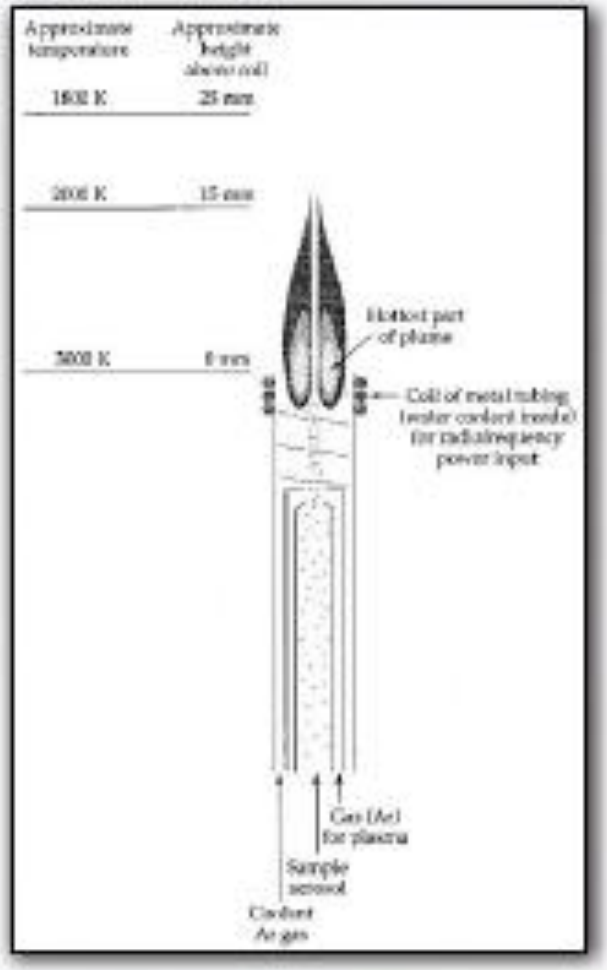
Field Desorption

This is an extension of the Field Evapouration process. One used the same type of needle, wherein micro-protrusions create large electric fields that can extract electrons to form ions by quantum tunneling process.

The difference is that the tip is removed from the vacuum chamber, dipped into the analyte solution, the analyte allowed to dry on the tip, and the tip is then reinserted into the vacuum chamber.

A high potential is applied, sometimes with gentle heating. The large field desorbs and ionizes the analyte. Spectra are quite simple, often producing only the parent ion peak, with no fragmentation at all.

Inductively Coupled Plasma

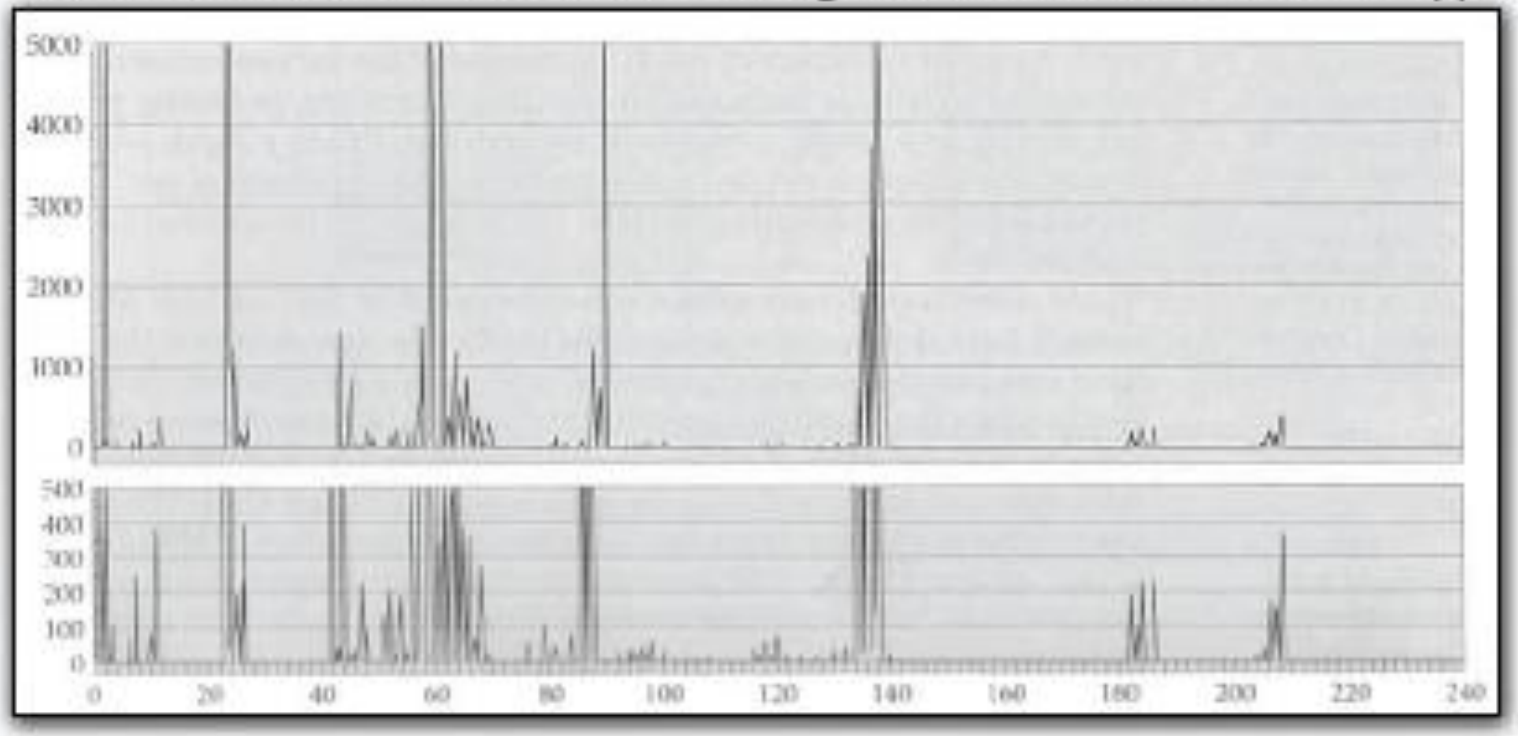


The ICP source is actually the least gentle – most aggressive – of sources. The fragmentation is so complete that everything is atomized. Temperatures inside the ICP torch can reach from 3000 - 6000 K. Everything is atomized and ionized – a plasma.

IC plasmas can be ionized by UV-Visible emission spectroscopy, but that can also be fed into the injection port of a mass spectrometer. Usually called ICP/MS.

Quantitative/Qualitative ICP/MS

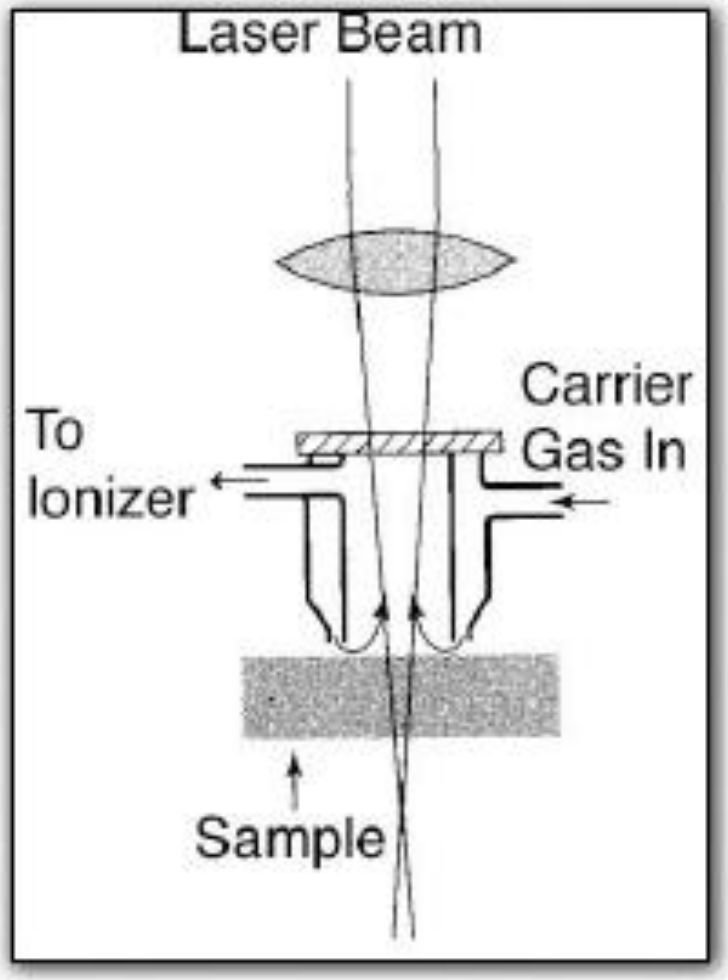
Here is a mass spectrum of a solution that contains a mixture of several different metals and non-metals. Scaling is such that 3000 counts ≈ 10 ppb.



Four heaviest clusters show isotope distribution for Pb, W, Ba, and Sr respectively. About 1 ppb of Pb and W, about 50 ppb of Sr and about 100 ppb of Ba.

Laser Ablation

High power, pulsed lasers can ablate the surface of solids that are not easily solvated otherwise. Surface heated at rate ~ 10^6 K/s, which blows atoms, molecules, and clusters from the surface. A carrier gas can move these particles into an ionizer, such as a ICP/MS for final analysis.



Matrix Assisted Laser Desorption Ionization - MALDI

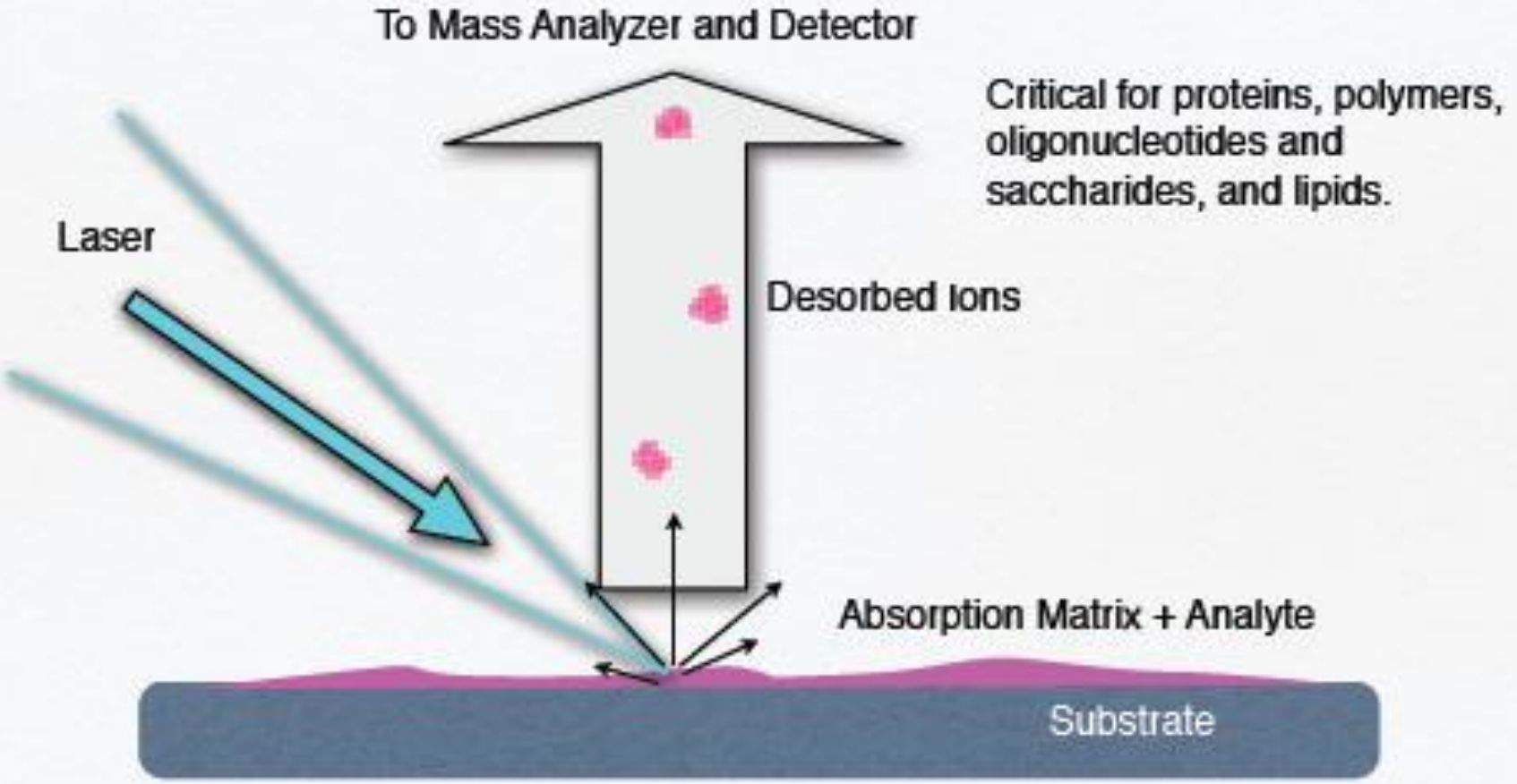
Rather new technique. Embed sample in a solid matrix (drying solution). The matrix contains a strongly absorbing species at a wavelength well away from where the analyte absorbs.

Sample is dramatically heated by a laser pulse absorbed by the matrix. This "blasts" a small "puff" of ionized sample into the vapour phase. It is extracted, accelerated, and focused into the mass analysis region.

Many matrices have now been identified. Analyze proteins with masses of several 100,000 amu.

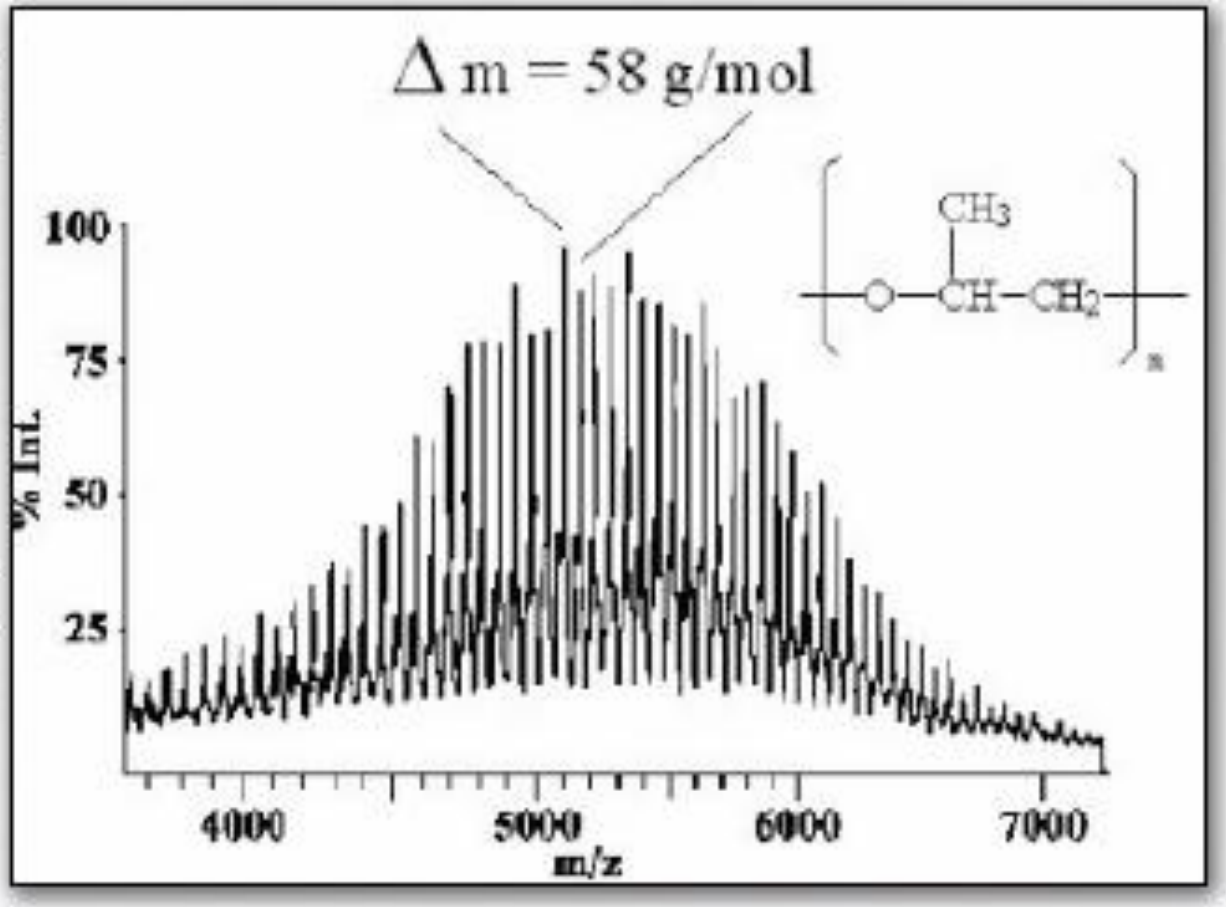
Nicotinic Acid	266 nm
2,5-dihyroxybenzoic acid	266 nm
Vanillic Acid	266 nm
2-aminopyrazine-2-carboxylic acid	337 nm
Caffeic acid	266 nm

MALDI Technique



MALDI Spectrum

Poly(Propylene Glycol) spectrum. Formed in a matrix of 2,5-dihydroxybenzoic acid in a 1:1 water:ethanol solvent. Note the broad distribution of masses. The peak mass is 5280 Da which is about 91 monomer units. The distribution is seen to be quasi-gaussian with a FWHM of about ±12 units.



Fast Atom Bombardment

Heat and light are only two ways in which sufficient energy can be deposited in a sample to vapourize and ionize the constituents. Atoms with high kinetic energy can do the same thing.

A high energy Ar^{+} ion beam is accelerated to 3 - 10 keV. The ions are neutralized in flight by a resonant electron exchange process with a cloud of Ar atoms. This high energy neutral atom beam strikes the surface, knocking off and ionizing substrate components. They are extracted, accelerated, and focused into the mass analyzer.

Solid substrate can be used. Advantages with a viscous, liquid substrate such as glycerol; the surface damage is immediately healed by the solvent flowing to fill pits formed by sputtering. Liquid matrix also reduces lattice energy – ions escape more easily.

Technique minimizes fragmentation. Measured masses over 10,000 Da.

Ion Sputtering

Similar to FAB is ion sputtering, the difference being that the accelerated ion beam is not neutralized before striking the surface. The ion beam can be produced as a focused beam which can be rastered across the surface for imaging, or it can arise from a glow discharge, which is an RF or DC discharge in an atmosphere of Ar. Electrostatic or magnetic fields direct the ions to the target surface. A third process, somewhat obscure, uses high energy He ions which are the decay products from a radioactive source.

Penning ionization is the mechanism by which the Ar^+ ions generate analyte ions during the surface collision.

Mass Analyzers

At the heart of a mass spectrometer is the mass analysis system. The selection is always based upon a mass-to-charge ratio, rather than on absolute mass. In many techniques, it is common to produce multiply charged species so that they show up at a much lower m/z value. The principal devices for mass selection are

- quadrupole (a quad)
- magnetic sector
- double focusing (sector)
- ion trap
- ion cyclotron
- time-of-flight (TOF)

Mass Resolution

A principal measure of the performance of a mass spec is its resolution. It is usually given as

$$R = \frac{m}{\Delta m}$$

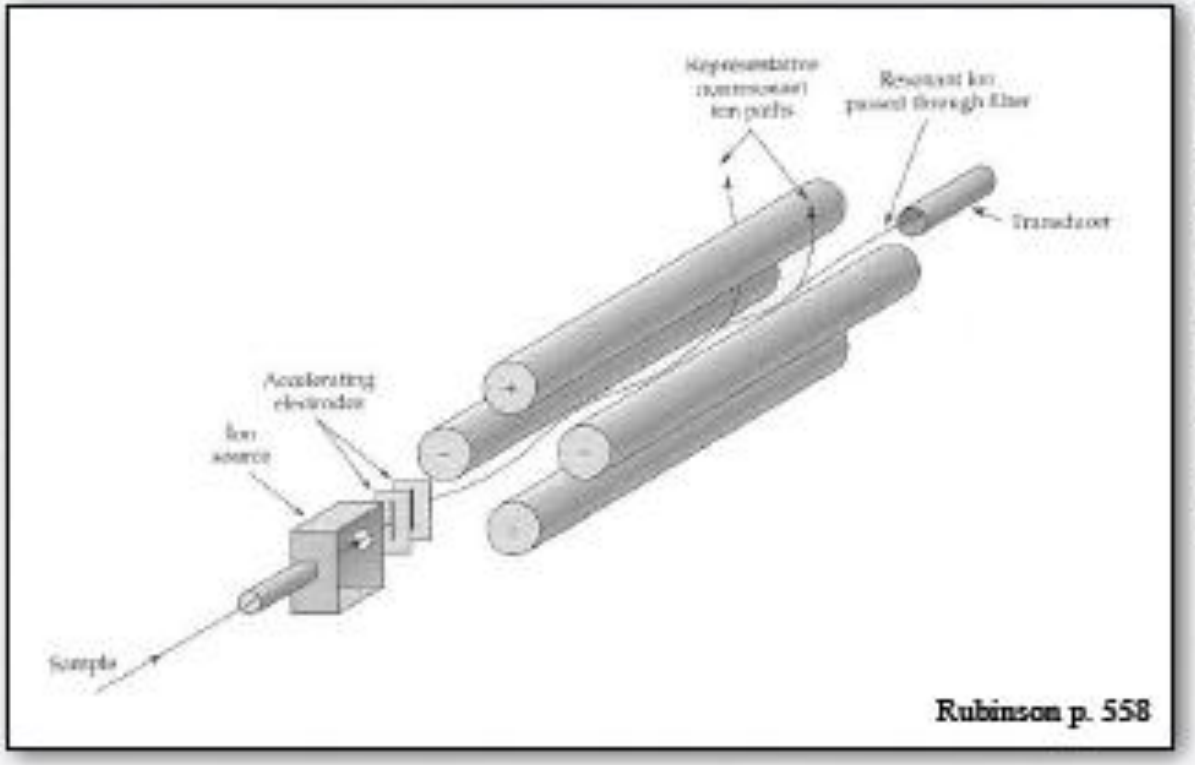
A spectrometer with a resolution of 2500 is needed to be able to distinguish between carbon monoxide (CO) and nitrogen (N_2). Both are nominally with a mass of 28 amu, but a more precise calculation has m(CO) = 27.9949 amu and m(N_2) = 28.0061 amu.

A resolution of 50 is all that is needed to distinguish ammonia (NH_3) from methane (CH_4) whose nominal masses differ by about 1 amu (17 amu vs. 16 amu).

Commercial units with resolution from 500 to 500,000 are available.

Quadrupole

Perhaps the most common mass spectrometer. Not the highest resolution. Opposite rods are polarized in phase with each other and out of phase with the other two rods.

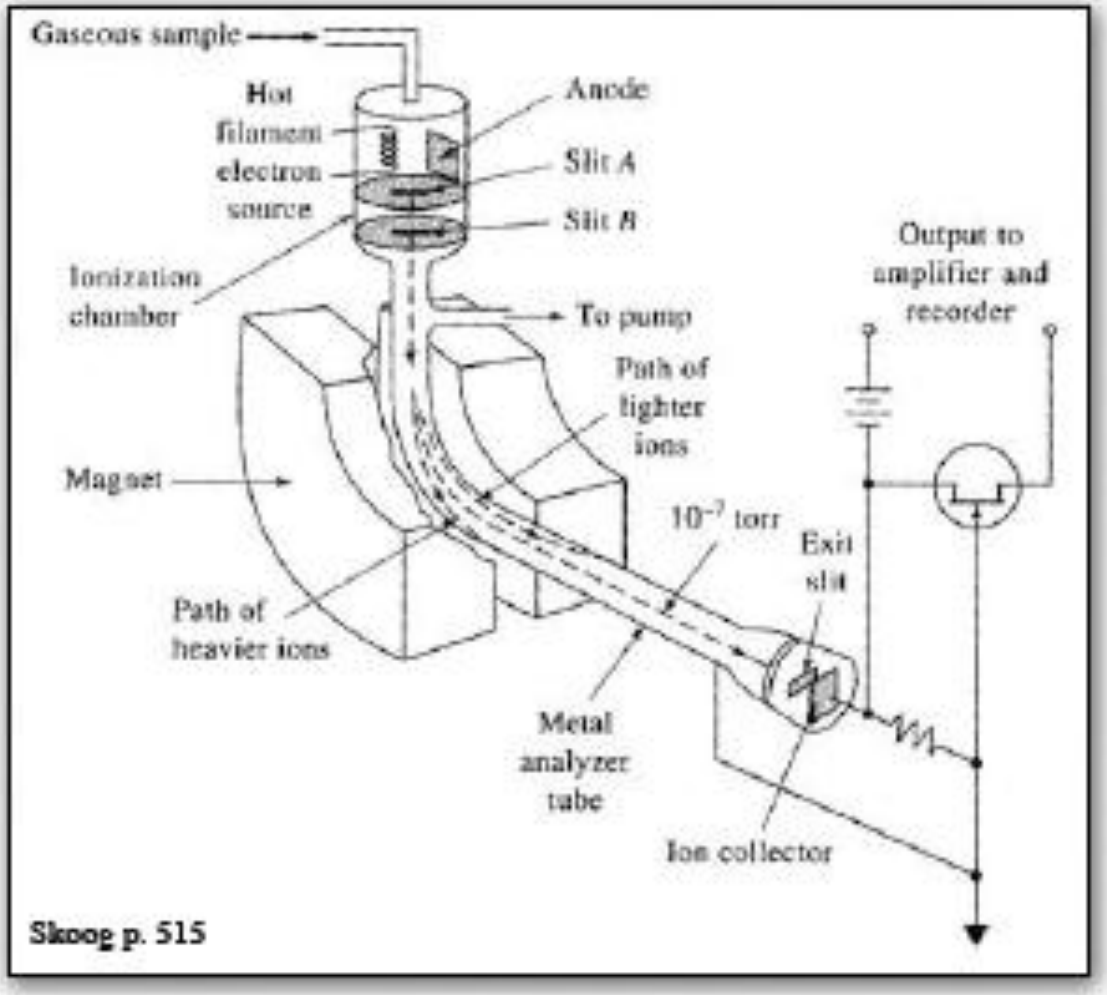


Rubinson p. 558

DC voltage drives ions to negative rods
RF signal reverses sign in time to push ions away.
Only specific m/z has right velocity to match with RF signal.
Scan masses by scanning RF and DC but keep ratio constant.

Magnetic Sector

A charged particle (an ion) experiences a force that bends its path when moving through a magnetic field. The balance between magnetic force and centripetal force brings an ion of a particular m/z to the entrance slit of a detector.



Skoog p. 515

Magnetic Sector Equations

The magnetic force F_m is given by

$$F_m = Bzev$$

This is balanced by the centripetal force, F_c

$$F_c = \frac{mv^2}{r}$$

These are equated and solved for the velocity

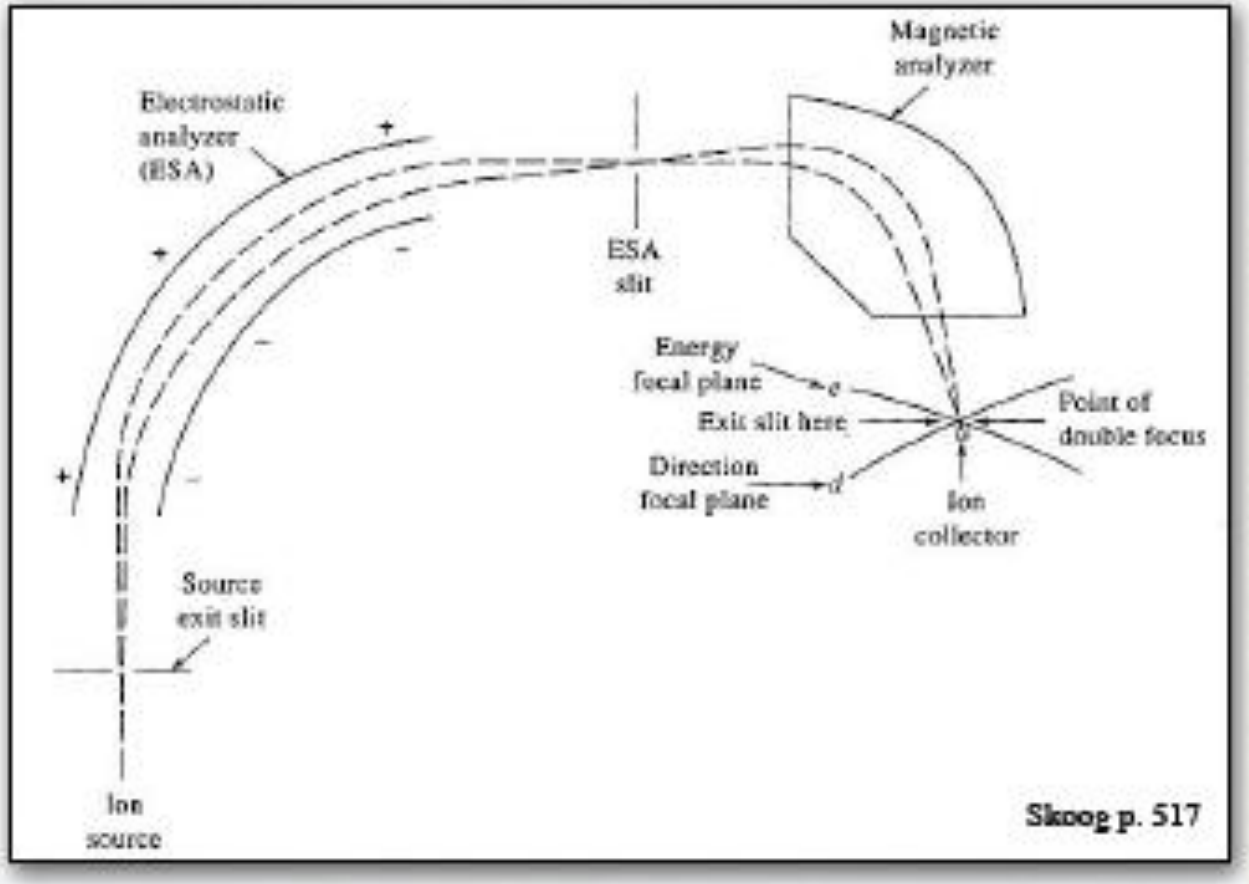
$$Bzev = \frac{mv^2}{r} \quad \Rightarrow \quad v = \frac{Bzer}{m}$$

Substitute into expression for kinetic energy (momentum and electrostatic). Solve for m/z.

$$E_k = zeV = \frac{1}{2}mv^2 = \frac{1}{2}m\left(\frac{Bzer}{m}\right)^2 \quad \Rightarrow \quad \frac{m}{z} = \frac{B^2 r^2 e}{2V}$$

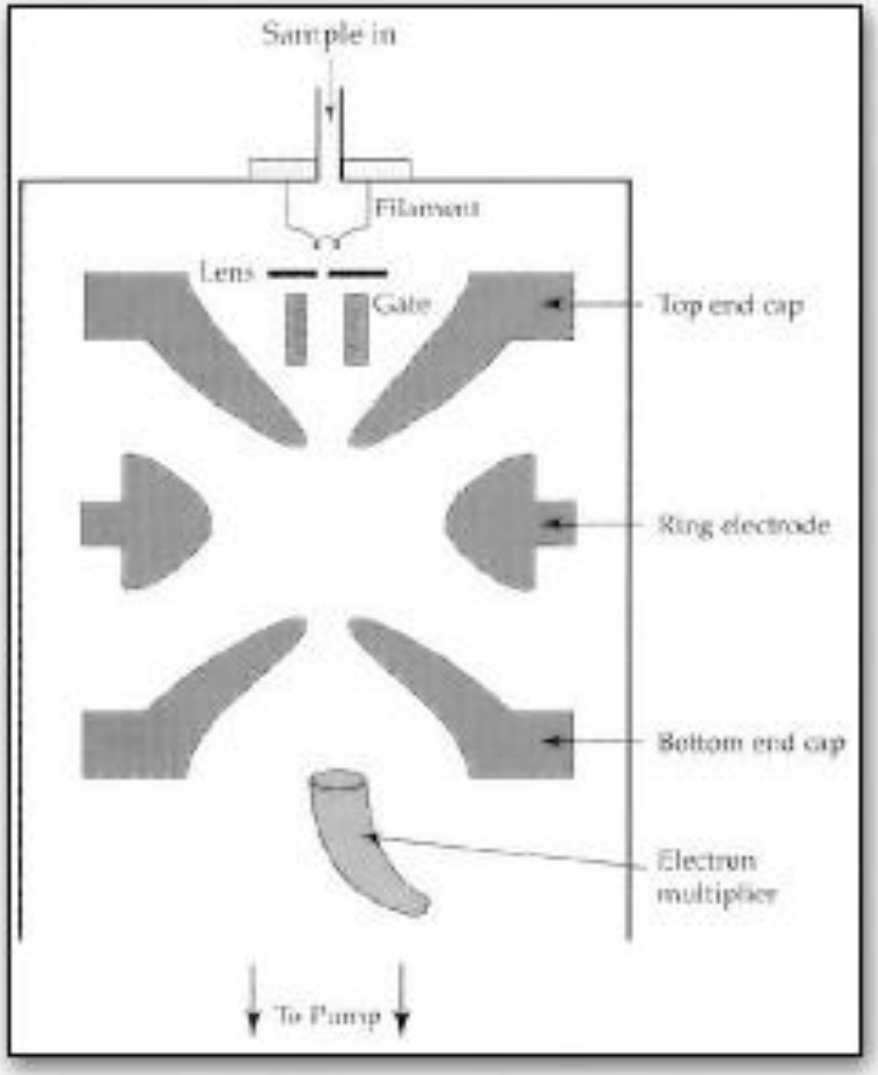
Double Focusing

A single magnetic sector instrument's resolution is limited by the spread of translational energy of the ions coming from the source. A double focusing instrument uses an electrostatic field to narrow the energy spread before the ions enter the magnetic sector. Resolutions of 10^5 are achievable with these instruments.

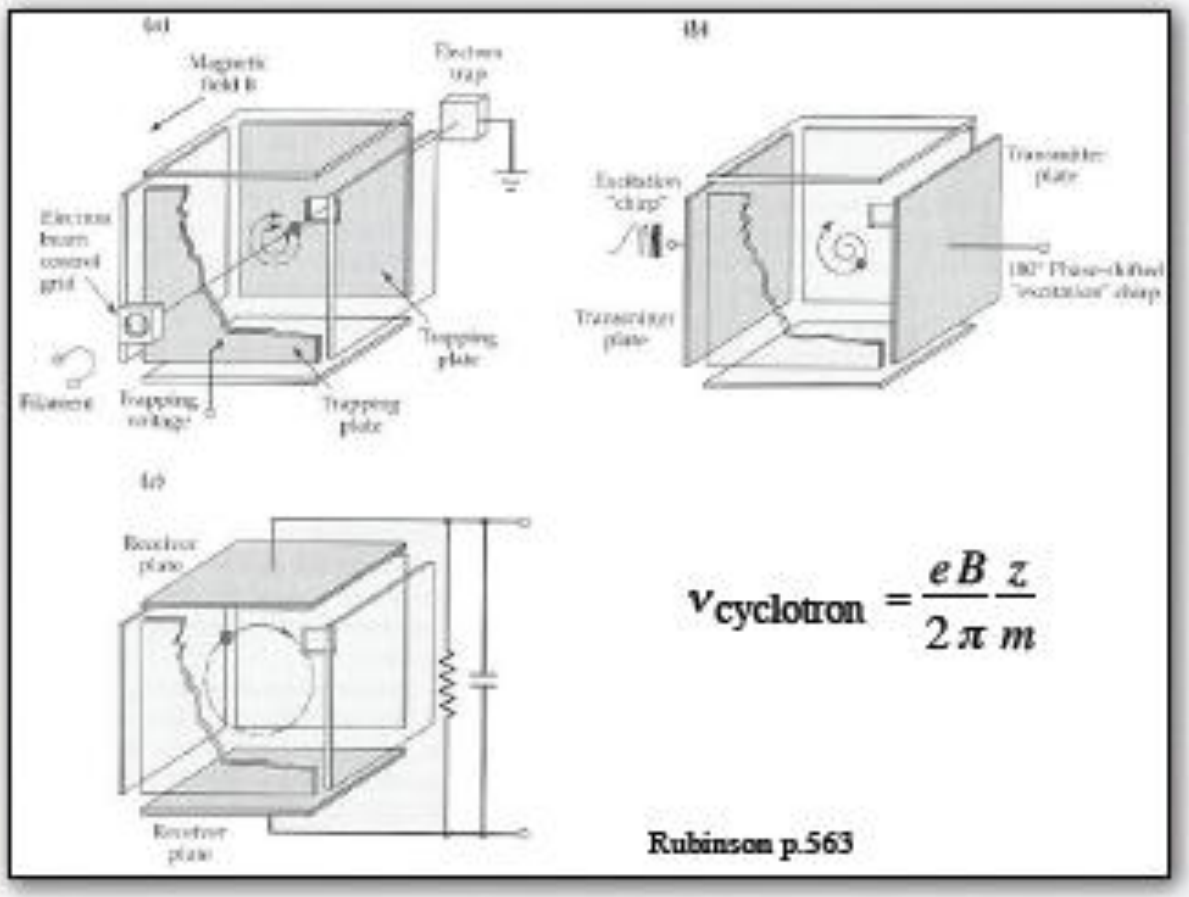


Ion Trap

This device is able to trap and hold ions in a space charge region with their masses extending over a range of several thousand Da. Adjusting the end cap fields to a particular frequency can drive a narrow Da range into the detector. Has a high ion yield for species. Dynamic concentration range is low. Ions spend a lot of time in trap and ion-ion reactions can alter the fragmentation pattern.



Ion Cyclotron



$$\nu_{\text{cyclotron}} = \frac{eB}{2\pi m}\frac{z}{}$$

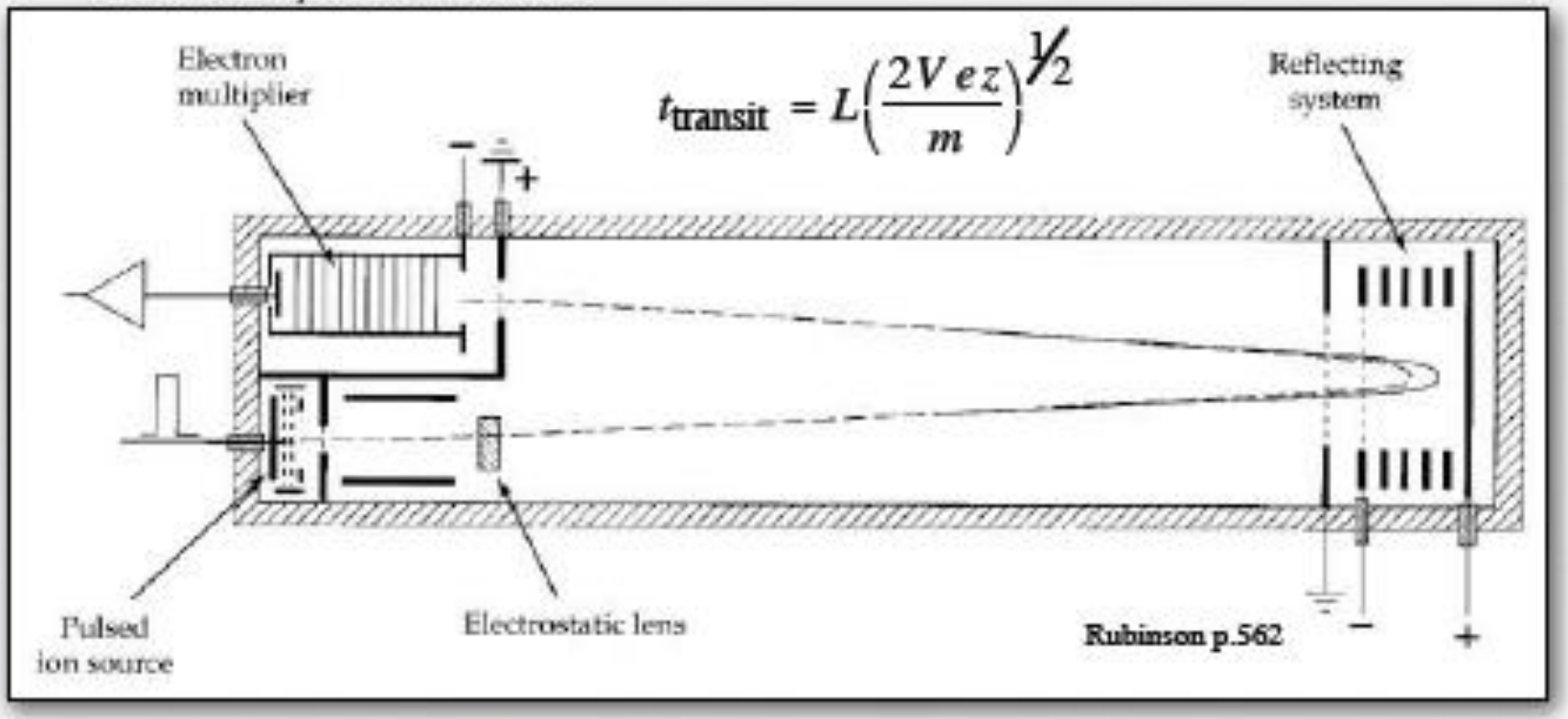
Rubinson p.563

Similar to in trap. Uses magnetic field and electric fields to induce circular motion. RF chirp excites circulating ions into larger orbits. Their decay induces currents in receiver coils. Time signal is FT to produce spectrum.

Resolution over 10^6. Detection limits in attomole range. Mass accuracy in ppb range.

Time of Flight (TOF)

Ions accelerated to same energy. Mass differences mean different velocities. They travel a long path (1 - 2 m) and arrive at the detector at different times. Time of arrival calculates m/z. Newer instruments are Reflectrons, with ion mirror.



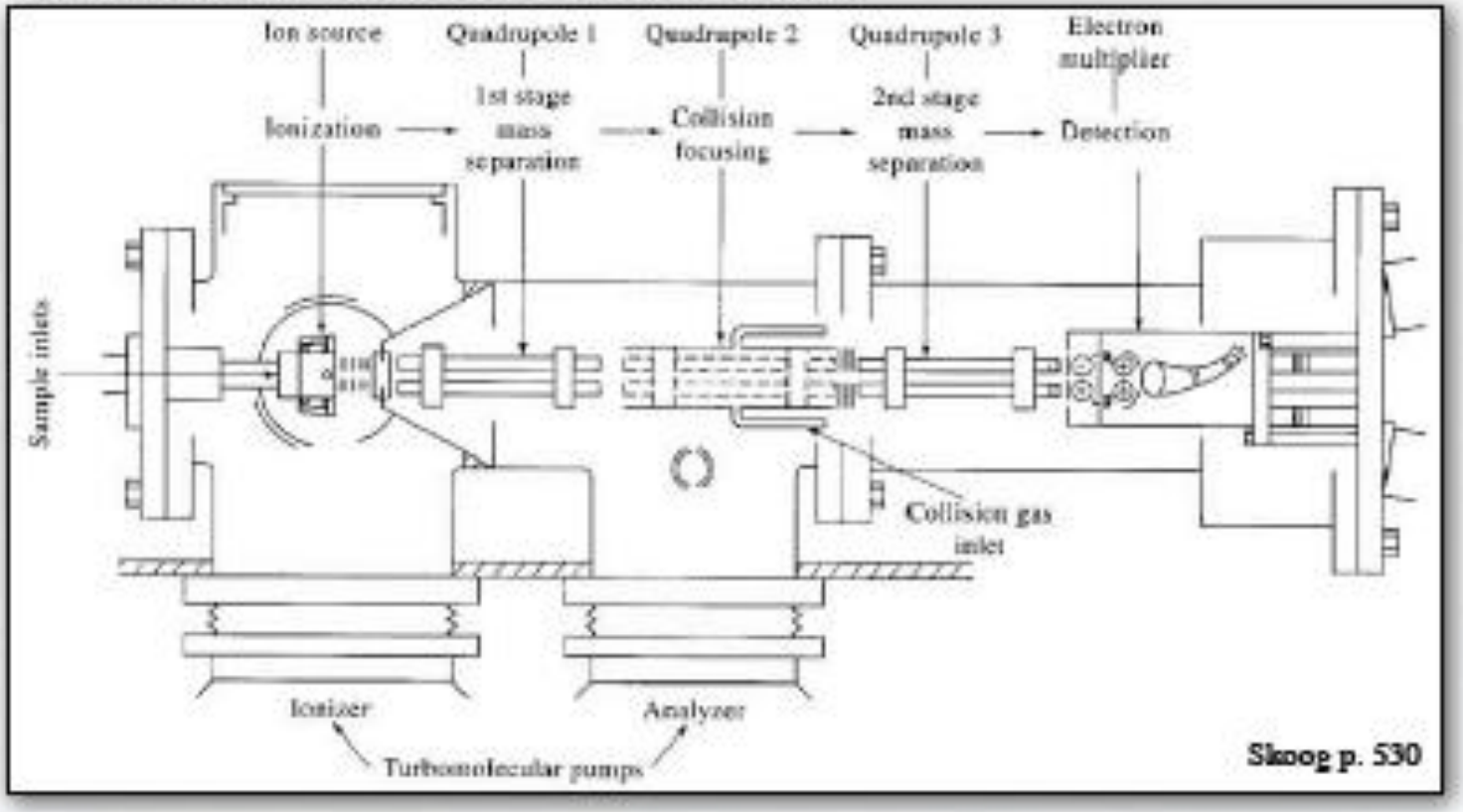
Tandem Mass Spectrometry

Usually referred to as MS/MS. Here one can take a mass spectrum of a mass spectrum. A particular ion fragment isolated in the first stage is directed to the second phase for further fragmentation and dispersion. For large biomolecules, this is essential for spectral analysis. There are three common types:

- EBEB: Double Double Focusing. Two double focusing magnetic sector stages.
- QQQ: A Triple Quad. Three quadrupole sections in series.
- Q-ToF: A quadrupole stage preceding a time-of-flight analyzer.

Triple Quad

(This is not a figure skating maneuver.) The second quad stage is not a mass separating quad but rather is a focusing quad which provides further collisional ionization with a He background gas.



Detectors

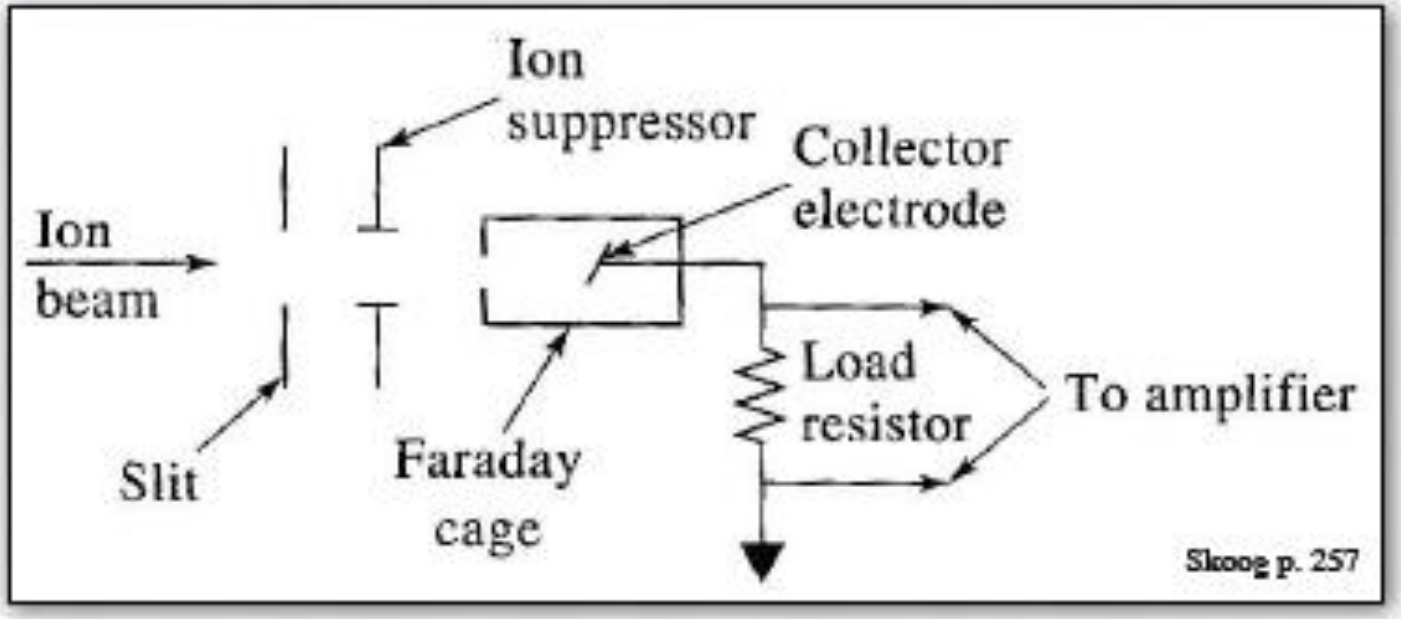
Detectors do not seem to have as many choices as with other techniques. Either measure current to neutralize charge or use impact kinetic energy to induce changes at detector.

- Faraday cup
- Electron multiplier
  - Channeltron
- Photographic plate
- Scintillation detector

Faraday Cup

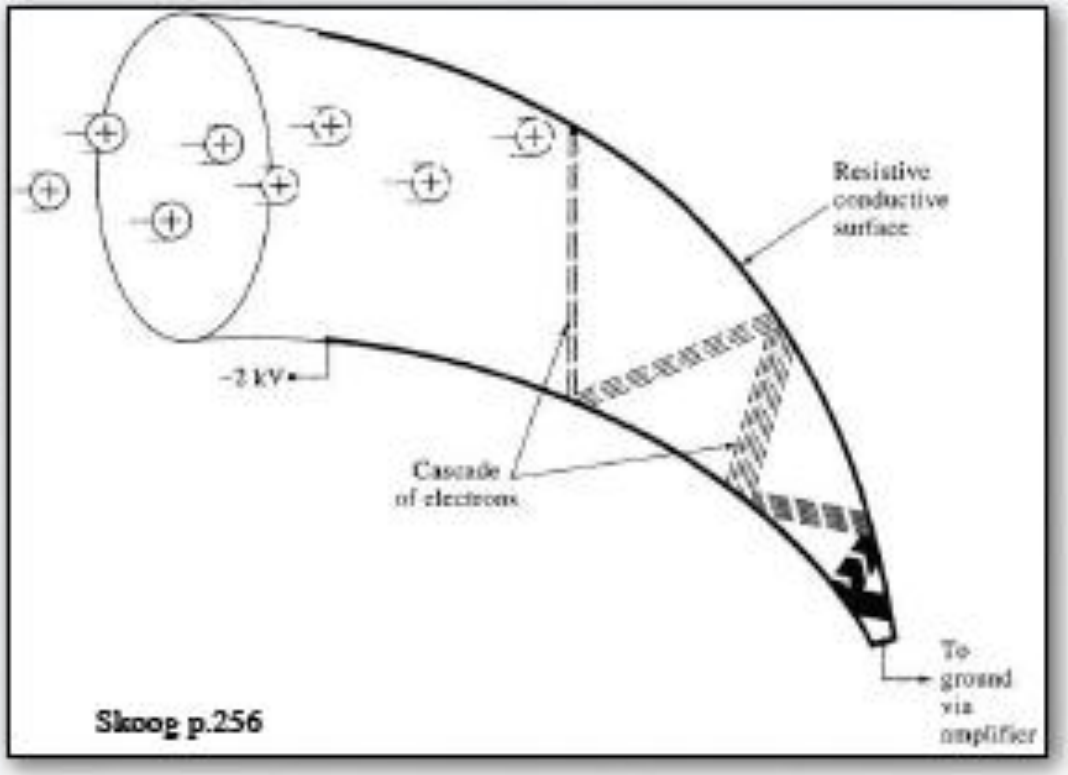
When ions strike a grounded metal plate, they are neutralized by drawing electrons from the metal. The metal plate replenishes its supply of electrons by drawing a current from ground. Measuring that current directly measures the ion current.

Effective if ion current is comparatively large. Very rugged and inexpensive.



Electron Multipliers

These are just like the PMTs used in optical spectroscopy. The incident ion beam ejects – because of its kinetic energy – electrons. By a chain of voltages on a series of dynodes, the electrons bounce towards ground, ejecting additional electrons at each stage. Gains from 10^5 to 10^8 can be realized. A trumpet shaped continuous dynode often called a Channeltron is a common implementation of these ideas.



Hyphenated Experiments

When a sample is complex – consisting several components, many whom may be large – such as is often found in environmental or biological environments, a mass spectrum becomes uninterpretable because of the plethora of peaks that arise. These complex systems are best handled by front-ending a mass spectrometer with a chromatographic separation scheme.

Output of chromatography column passes directly into electrospray ionizer.

- GC-MS
- GC-MS-MS
- LC-MS

Capillary Electrophoresis is proving invaluable for protein and DNA analysis when coupled with a mass spectrometer.

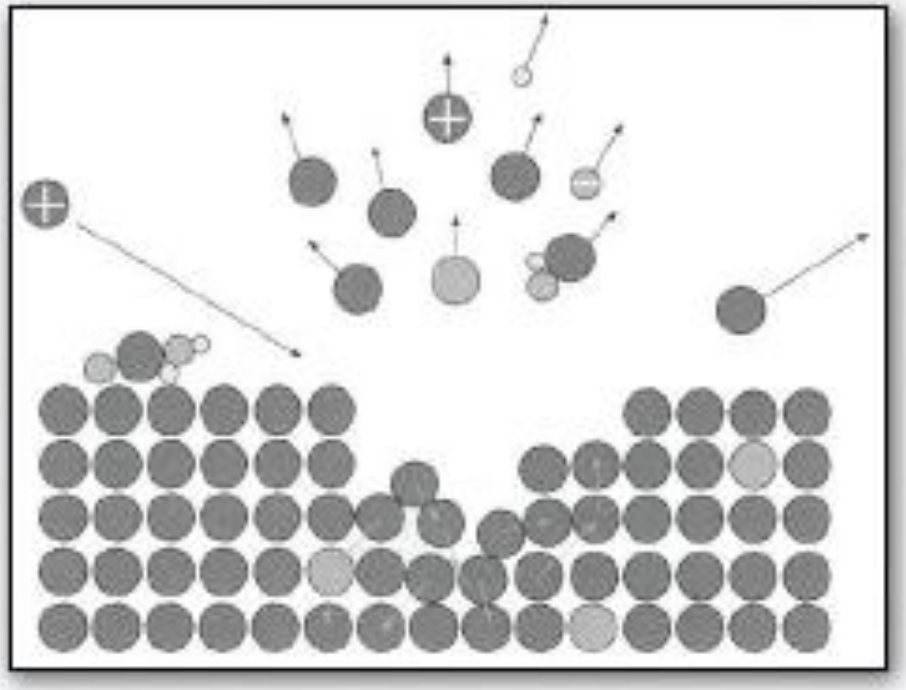
- CE-MS

Spatial Analysis

Sputtering ion beams can be used to analyze a solid sample for the spatial distribution of properties. The most important technique is called Secondary Ion Mass Spectrometry (SIMS). It is one of the more sensitive techniques for determining the chemical nature of a surface.

There are three levels of information that can be obtained from a sample:

- Globally, what is the composition of the sample?
- How does the composition vary with position on the surface?
- How does the composition vary with depth into the surface?



Static SIMS (SSIMS)

When the incident ion current is very low (< 1 nA/cm^2), the sputtered ions can be collected and mass analyzed in a time that is short compared to the lifetime of the surface. Less than 1% of the surface has been eroded in order to take the spectrum. The name "static" is used to infer that the surface has not been changed.

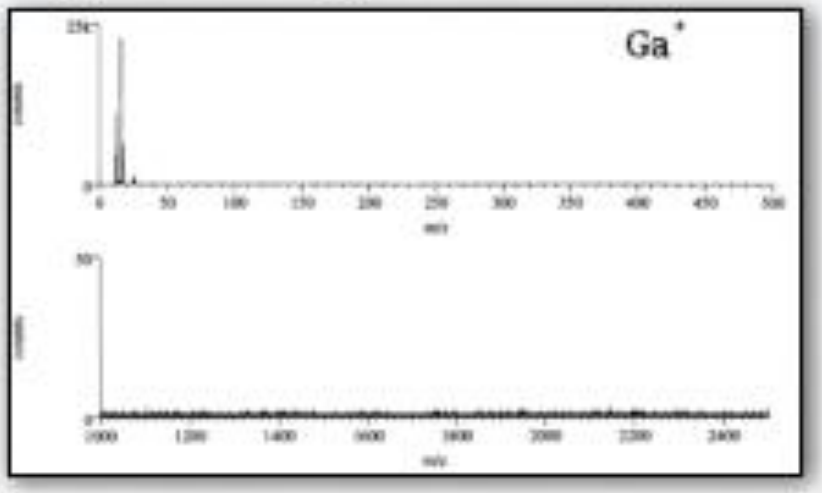
With newer ion guns, the ion beam can focused to as small a spot as a few nanometers. This ion beam can then be raster scanned across a surface. The mass spectrometer monitors a particular ion (say, Fe^+). A correlation between secondary ion current and beam position creates a chemical map of the surface.

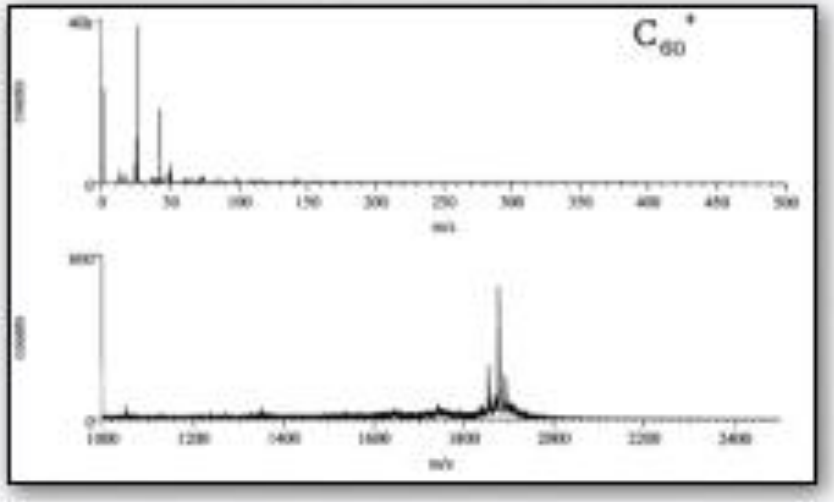
Bubert p.99

Damaged car paint. Silicone oil in paint (2). (Poly) fluoroether in paint chip. Contamination droplet lead to paint failure.

SIMS with Heavy Projectiles

A C_{60}^+ ion gun from Ionoptika.



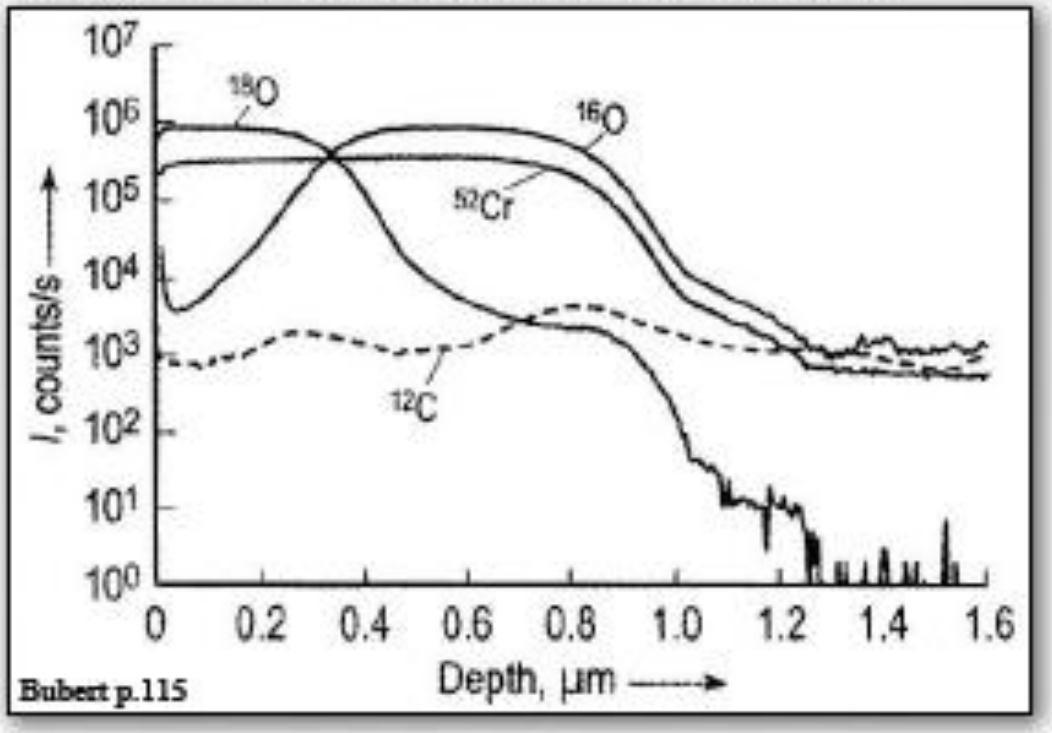


A heavier projectile of the same kinetic energy has less momentum. Also, the C_{60} molecule is larger than an atom and its collision energy is spread over a larger area of the surface. Less fragmentation is observed. Spectra of gramacidin D. Peak at 1881 amu is missing in Ga^+ spectrum but clearly present in C_{60}^+.

Dynamic SIMS (DSIMS)

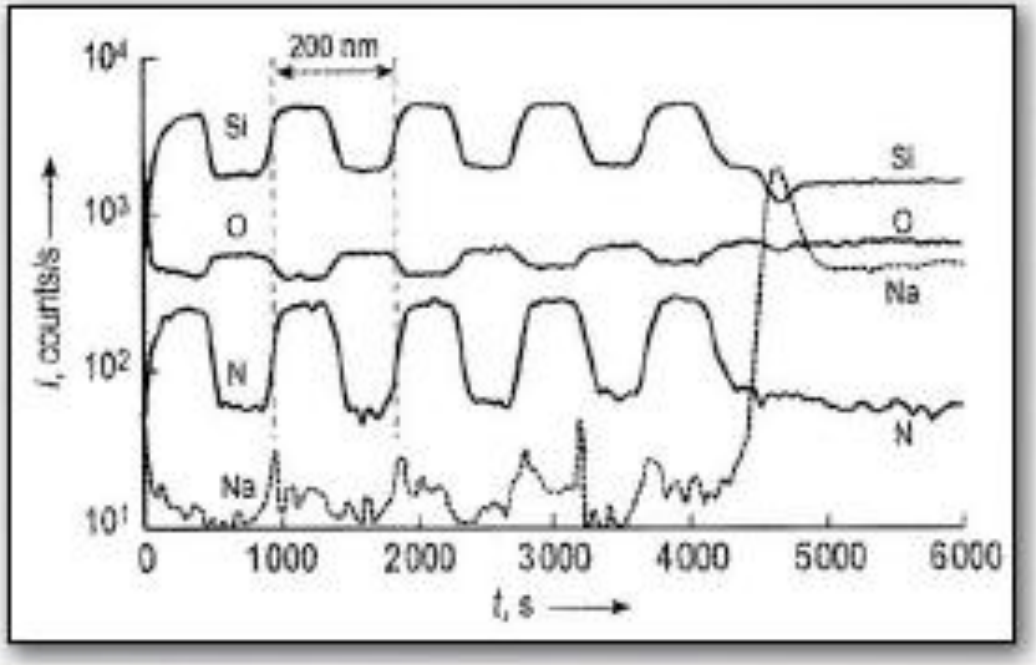
If we increase the incident ion beam current into the mA range, the surface now is quickly eroded; the ion beam chews up the surface a measurable rate. The word "dynamic" is meant to infer a changing surface, arising because of the sputtering process. Monitoring the spectrum as a function of time, correlates the composition with the depth into the surface. This is called depth profiling.

Oxidation of Cr. First with ^{16}O and then ^{18}O. Shows that Cr diffuses through existing oxide to further react at the surface.



Secondary Neutral Mass Spectrometry (SNMS)

If we do NOT look at the secondary ions, but rather collect the neutral molecules, and treat them to mass analysis (ionization, etc.). SNMS is more quantitative than SIMS because the ionization process is better understood and reproducible. Accurate surface compositions can be determined with this experiment, but sensitivity is lower by an order of magnitude.



Depth profiling. 100 nm of SiO_2 and 100 nm of Si_3N_4, alternating 5 times. They were grown on a glass substrate. Hence, the Na signal jumps up at the end.