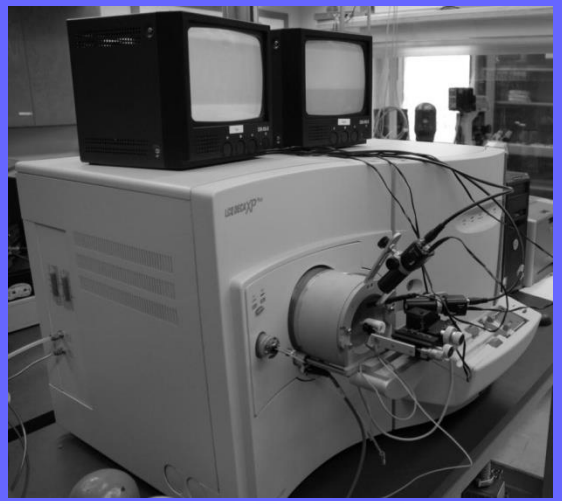
Chemistry 4631

Instrumental Analysis

Lecture 32



Mass Spectrometry (MS)

Instrumentation

Principle components:

- Inlet
- Ion source
- Mass analyzer
- **Ion transducer**
- Pumps
- Signal processor

Mass Spectrometry (MS)

Instrumentation

Transducers

Discrete dynode electron multiplier

- Most common
- Designed for detection of positive ions
- Similar to PMT
- Rugged
- High Current gain
- Nanosecond response times

Mass Spectrometry (MS)

Instrumentation

Transducers

Discrete dynode electron multiplier

Continuous dynodes held at successively higher voltages.

The cathode and dynode surfaces are coated with Cu/Be which emit electrons when struck by energetic ions or electrons.

Typically have ~ 20 dynodes with an overall gain of ~ 10^7.

Mass Spectrometry (MS)

Discrete dynode electron multiplier

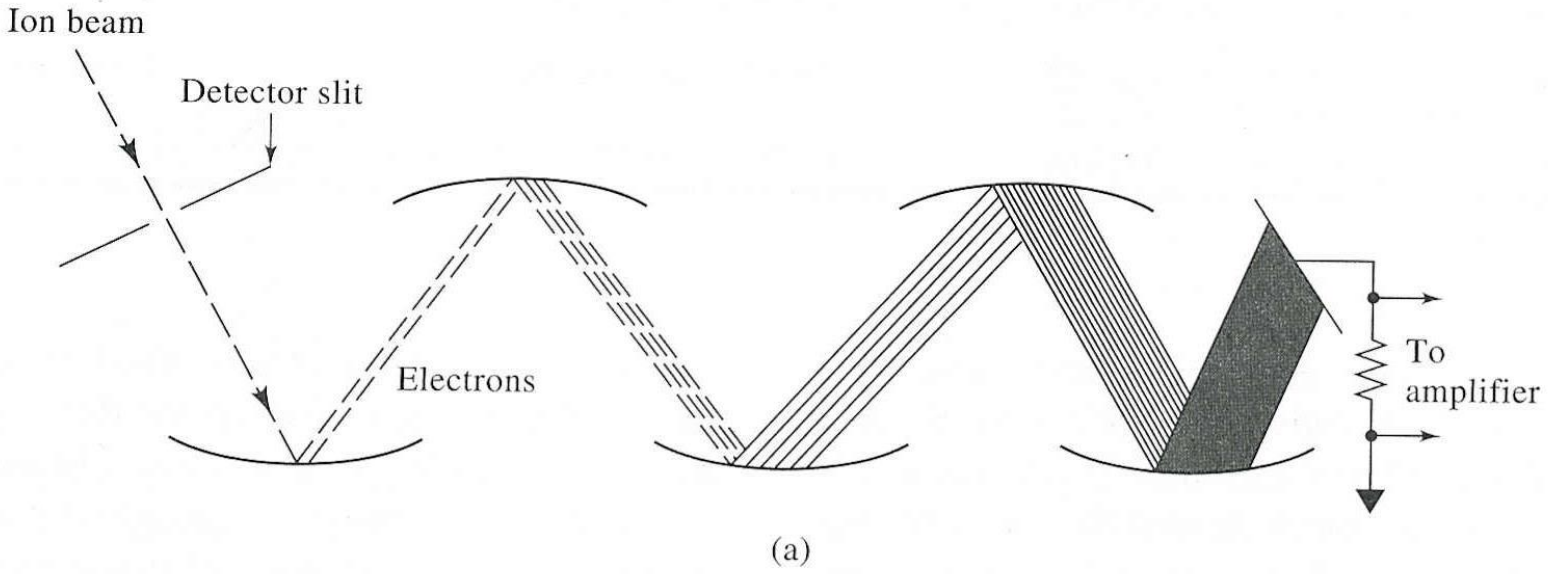


Figure 11-2 (a) Discrete dynode electron multiplier. Dynodes are kept at successively higher potentials via a multistage voltage divider. (b) Continuous dynode electron multiplier. *(Adapted from J. T. Watson,* Introduction to Mass Spectrometry, *p. 247. New York: Raven Press, 1985. With permission.)*

Mass Spectrometry (MS)

Instrumentation

Transducers

Continuous-dynode electron multiplier

Trumpet-shaped device made of glass doped with lead.

Ions emerging from mass analyzer are directed by a charge deflector plate into the detector.

A progressive potential of 1.8 - 2 kV is applied across the length of transducer.

Mass Spectrometry (MS)

Instrumentation

Transducers

Continuous-dynode electron multiplier

When an ion collides with the lead oxide coating on the detector walls, electrons are emitted.

With each strike on the detector walls the total emitted electrons are multiplied (amplification) and accelerated down the tube.

Gain ~ 10^5

Mass Spectrometry (MS)

Continuous-dynode electron multiplier

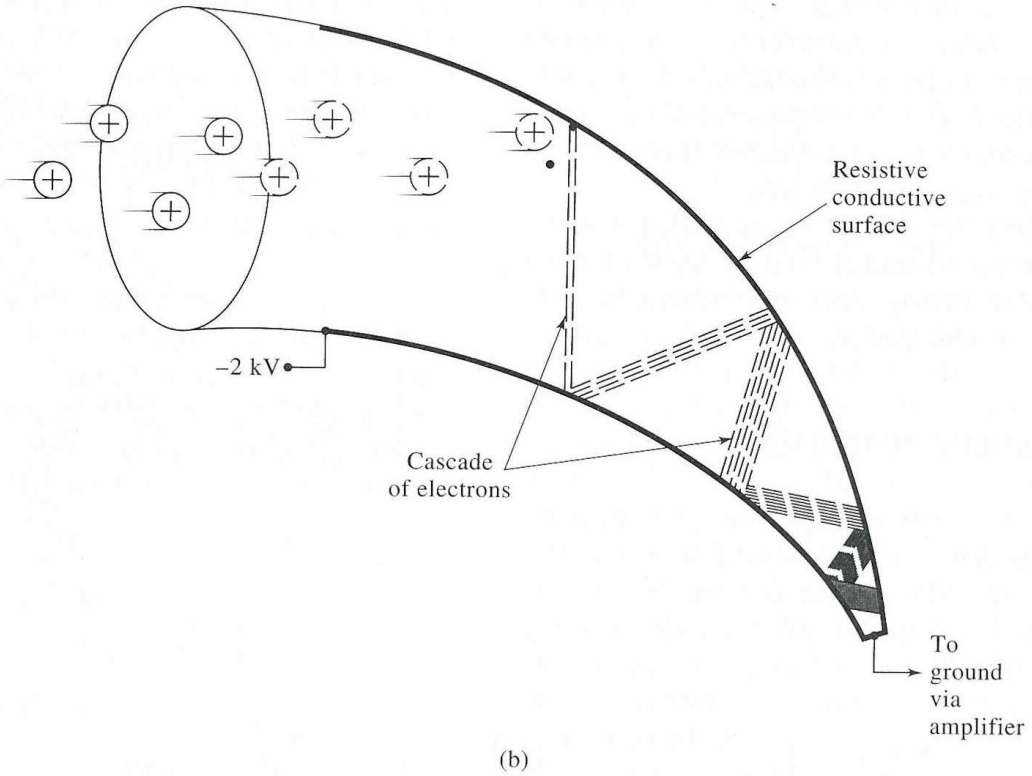


Figure 11-2 (a) Discrete dynode electron multiplier. Dynodes are kept at successively higher potentials via a multistage voltage divider. (b) Continuous dynode electron multiplier. *(Adapted from J. T. Watson,* Introduction to Mass Spectrometry, *p. 247. New York: Raven Press, 1985. With permission.)*

Mass Spectrometry (MS)

Instrumentation

Transducers

Faraday Cup

Ions strike a collector electrode surrounded by a cage to prevent escape of reflected ions or secondary electrons.

The collector and cage are connected to ground through a large resistor.

Charge of positive ions striking plate is neutralized by flow of electrons from ground through the resistor.

The resulting potential drop across the resistor is amplified.

Mass Spectrometry (MS)

Faraday Cup

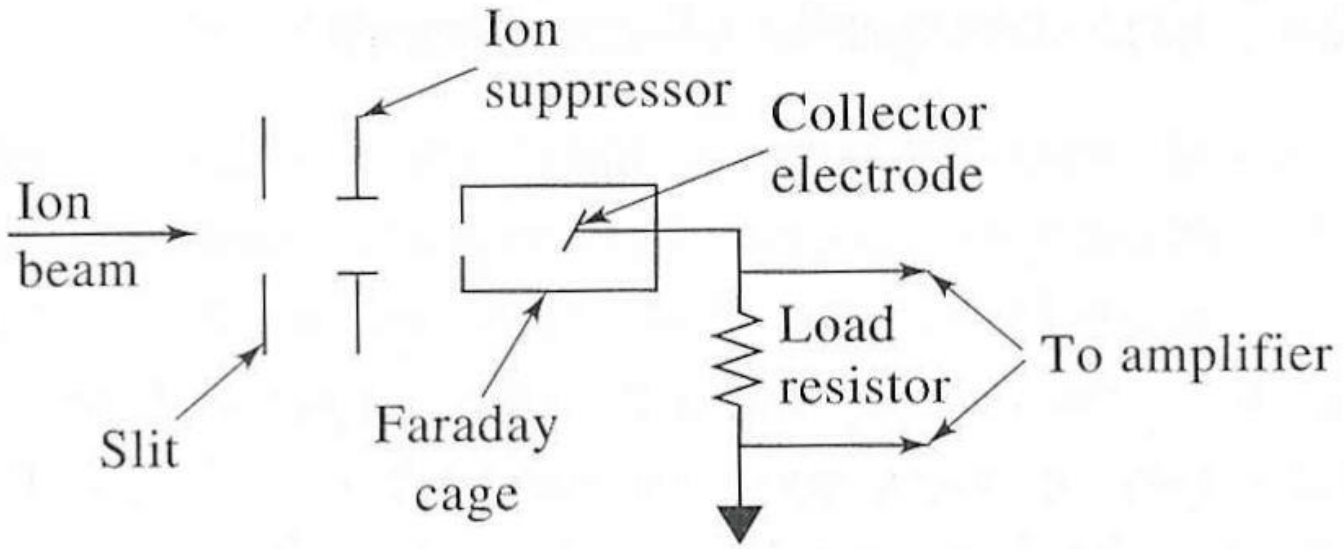


Figure 11-3 Faraday cup detector. The potential on the ion suppressor plates is adjusted to minimize differential response as a function of mass.

Mass Spectrometry (MS)

Instrumentation

Transducers

Faraday Cup

Response is independent of energy, mass, chemical nature of the ions.

Advantages

- **Inexpensive**
- **Simple mechanically and electrically**

Disadvantages

- **Speed is limited by high-impedence amplifier**
- **Less sensitive since no internal amplification**

Mass Spectrometry (MS)

Most analytes separated by HPLC are thermally stable and non-volatile (liquids) (unlike in GC) – so not ionized easily by EI or CI techniques.

MS must be at 10^{-6} torr

Mass Spectrometry (MS)

Instrumentation

Detectors

Liquid Chromatography / Mass spectrometry

- Advantages:
- More definitive identifications
- Wide range of analytes can be studied
- Sensitivity (pg)

Mass Spectrometry (MS)

Instrumentation

Detectors

LC-MS

- **Problems for LC-MS combination:**
- **HPLC mobile phase – liquid w/ water or organics**
- **MS must be at 10^{-6} torr**
- **Most analytes separated by HPLC are thermally stable and non-volatile (unlike in GC) – so not ionized easily by EI or CI techniques**

Mass Spectrometry (MS)

Instrumentation

Detectors - LC-MS

- Ideal Interface:
- Has no reduction in chromatographic performance
- No chemical modifications
- High sample transfer
- Reliable and reproducible

Mass Spectrometry (MS)

Instrumentation

LC-MS

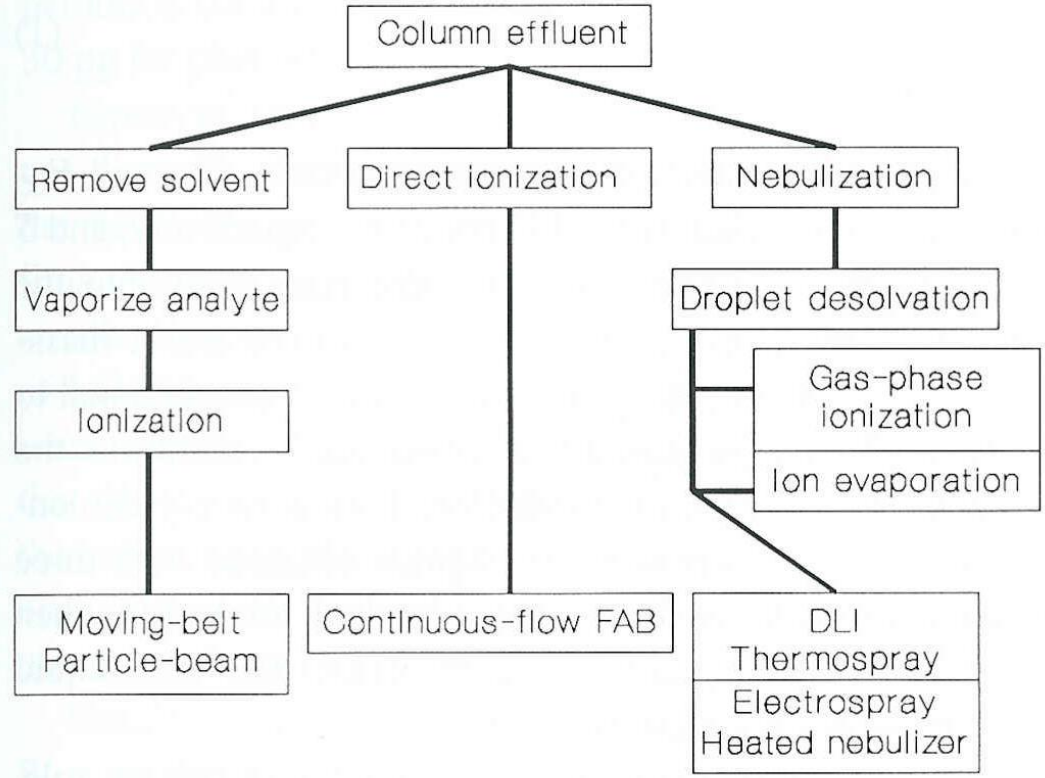


Fig. 1.5. Three general strategies to LC-MS interfacing.

Mass Spectrometry (MS)

Instrumentation

Ion source

- Moving-belt 1977
- Direct-liquid-introduction 1980
- Thermospray 1983
- Frit FAB/continuous-flow FAB 1985/1986
- Atmospheric-pressure chemical ionization 1986
- Particle-beam 1988
- Electrospray 1988

Mass Spectrometry (MS)

Instrumentation

Electrospray 1988

- A liquid, in which the analyte(s) of interest have been dissolved, is passed through a capillary (typically stainless steel), at atmospheric pressure, maintained at high voltage (3 to 4 kV).

- The liquid stream breaks up with the formation of highly charged droplets which are desolvated as they pass through the atmospheric-pressure region of the source towards a counter electrode.

Mass Spectrometry (MS)

Instrumentation

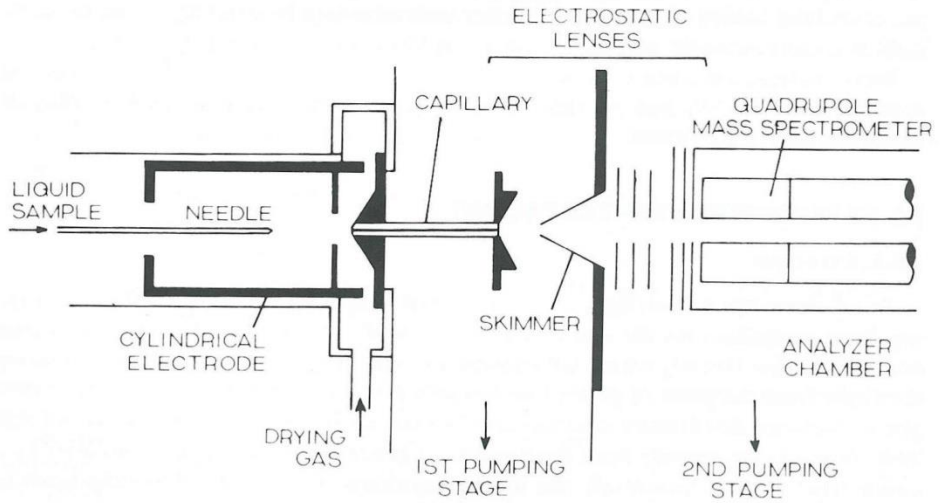
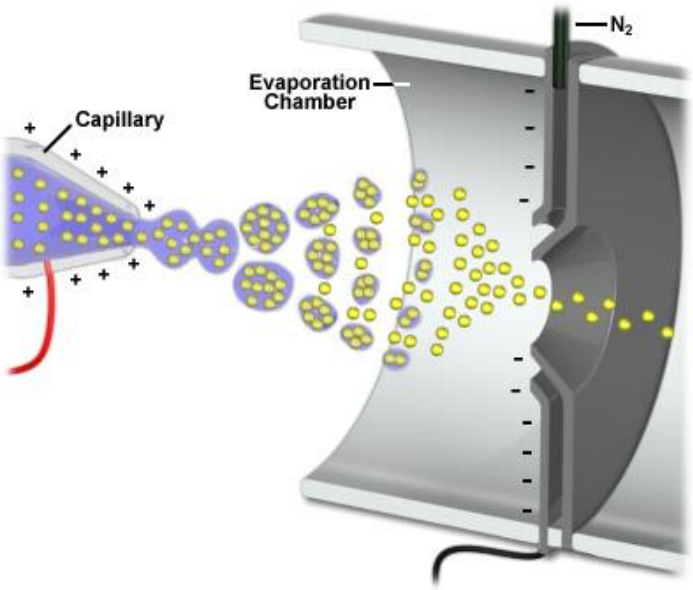


Fig. 1.13. Schematic diagram of the first-generation electrospray LC-MS interface as described by Whitehouse et al. [216]. Reproduced from Ref. [216] with permission. © 1985, American Chemical Society.



Mass Spectrometry (MS)

Instrumentation

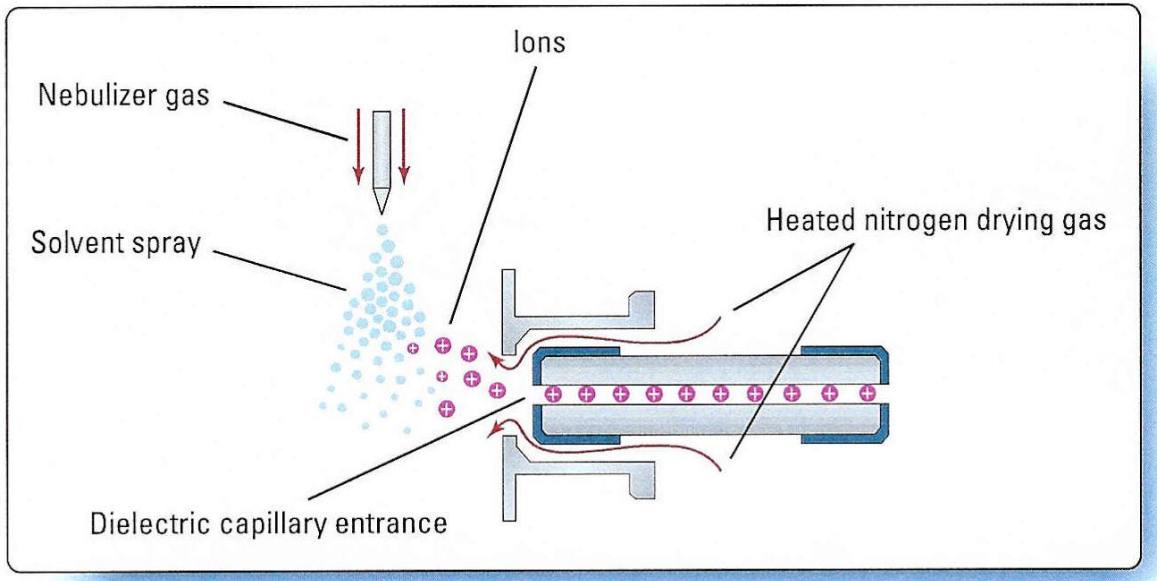


Figure 4. Electrospray ion source

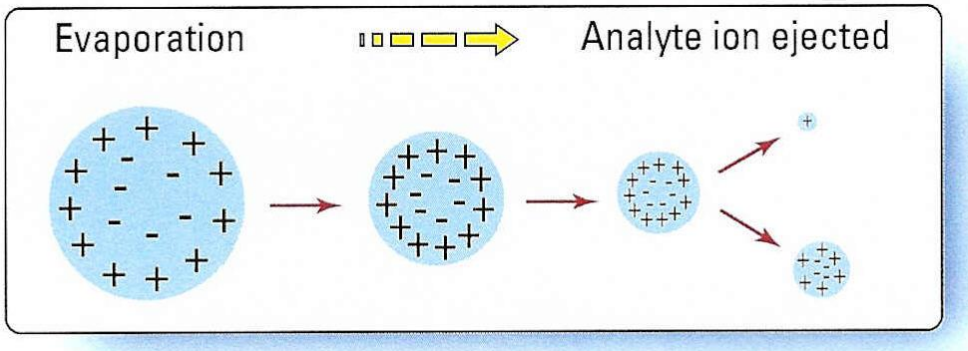


Figure 5. Desorption of ions from solution

Mass Spectrometry (MS)

Electrospray

- **Desolvation is assisted by a stream of a drying gas, usually nitrogen, being continually passed into the spraying region.**
- **As the droplets shrink, the charge concentration in the droplets increases. The repulsive force between ions with like charges exceeds the cohesive forces and ions are ejected (desorbed) into the gas phase.**
- **Analyte ions are obtained from these droplets which then pass through two differentially pumped regions into the source of the mass spectrometer.**

Mass Spectrometry (MS)

Electrospray

- **Since ionization takes place directly from solution, thermally labile molecules may be ionized without degradation.**
- **In contrast to most other ionization methods, the majority of ions produced by electrospray are multiply charged.**
- **Electrospray is useful for large biomolecules such as proteins, peptides, etc… while still able to analyze smaller molecules.**

Mass Spectrometry (MS)

Electrospray

In positive ionization mode:

a trace of formic acid is often added to aid protonation of the sample molecules.

In negative ionization mode:

a trace of ammonia solution or a volatile amine is added to aid deprotonation of the sample molecules.

Mass Spectrometry (MS)

Electrospray – Disadvantages

- **Electrospray is not applicable to non-polar or low-polarity compounds.**
- **The mass spectrum produced from an analyte depends upon a number of factors and spectra obtained using different experimental conditions may therefore differ considerably in appearance.**
- **Suppression effects may be observed and the direct analysis of mixtures is not always possible. This has potential implications for co-eluting analytes in LC–MS.**

Mass Spectrometry (MS)

Electrospray – Disadvantages

- **Electrospray is a soft-ionization method producing intact molecular species and structural information is not usually available.**

- **Electrospray sources are capable of producing structural information from cone-voltage fragmentation but these spectra are not always easily interpretable. Experimentally, the best solution is to use a mass spectrometer capable of MS–MS operation but this has financial implications.**

Mass Spectrometry (MS)

Electrospray – Advantages

- **Ionization occurs directly from solution and consequently allows ionic and thermally labile compounds to be studied.**

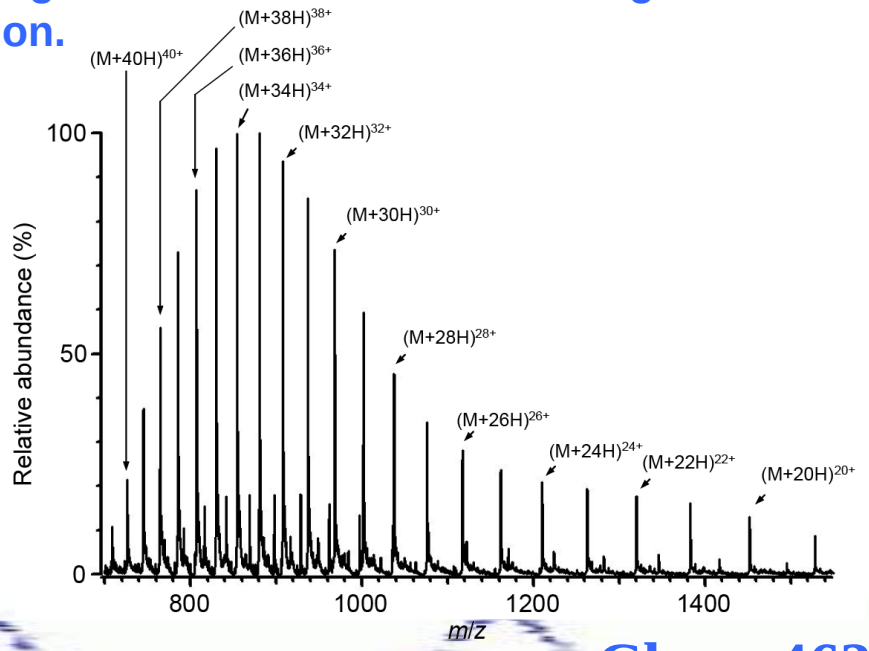
- **Mobile phase flow rates from nl min^{-1} to in excess of 1 ml min^{-1} can be used with appropriate hardware, thus allowing conventional and microbore columns to be employed.**

Mass Spectrometry (MS)

Electrospray – Advantages

- Electrospray ionization, in contrast to the majority of other ionization methods, produces predominantly multiply charged ions of the intact solute molecule. This effectively extends the mass range of the mass spectrometer and allows the study of molecules with molecular weights well outside its normal range.

- For high-molecular-weight materials, an electrospray spectrum provides a number of independent molecular weight determinations from a single spectrum and thus increased precision.



Mass Spectrometry (MS)

Ionization chemistry

For electrospray, formation of analyte ions in solution is essential to achieving good results.

Techniques to help ion formation include:

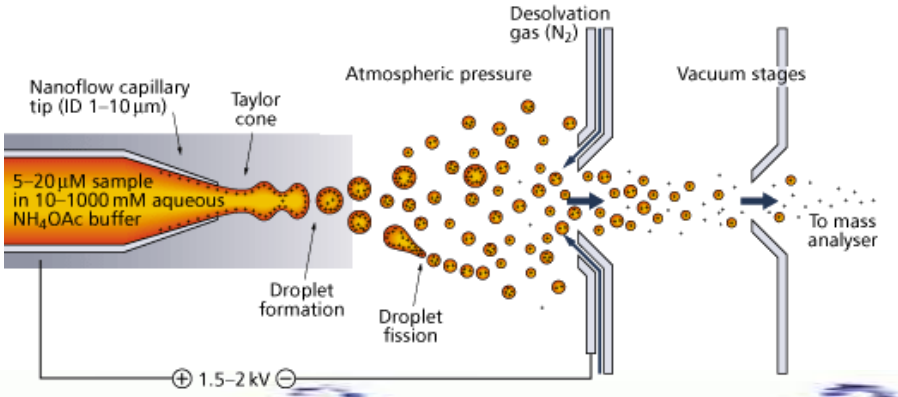
- **select more volatile buffers to reduce the buildup of salts in the ion source**
- **adjust solvent pH according to the polarity of ions desired and the pH of the sample**
- **use solvents that have low heats of vaporization and low surface tensions to enhance ion desorption**
- **make sure that gas-phase reactions do not neutralize ions through proton transfer or ion pair reactions**

Mass Spectrometry (MS)

Nanospray ionization - A low flow rate version of electrospray.

- **The flow rate of solute and solvent using this procedure is very low, 30 - 1000 nL/min, and so far less sample is consumed than with the standard electrospray ionization technique.**

- **A common application of this technique is for a protein digest mixture to be analyzed to generate a list of molecular masses for the components present, and then each component to be analyzed further by tandem mass spectrometric (MS-MS) amino acid sequencing techniques**



Mass Spectrometry (MS)

Tandem Mass Spectrometry (MS–MS)

Tandem mass spectrometry (MS–MS) is a term which covers a number of techniques where

one stage of mass spectrometry (not necessarily the first) is used to isolate an ion of interest and a

second stage is then used to probe the relationship of this ion with others from which it may have been generated or which it may generate on decomposition.

third stage – can have more than 2 – so can introduce holding stages, etc…

Mass Spectrometry (MS)

Since Tandem MS involves three distinct steps of selection-fragmentation-detection, the separation of these three steps can be realized in space or in time.

Tandem MS in space

Typical Tandem MS in space instruments include QqQ, QTOF, and hybrid ion trap/FTMS, etc.

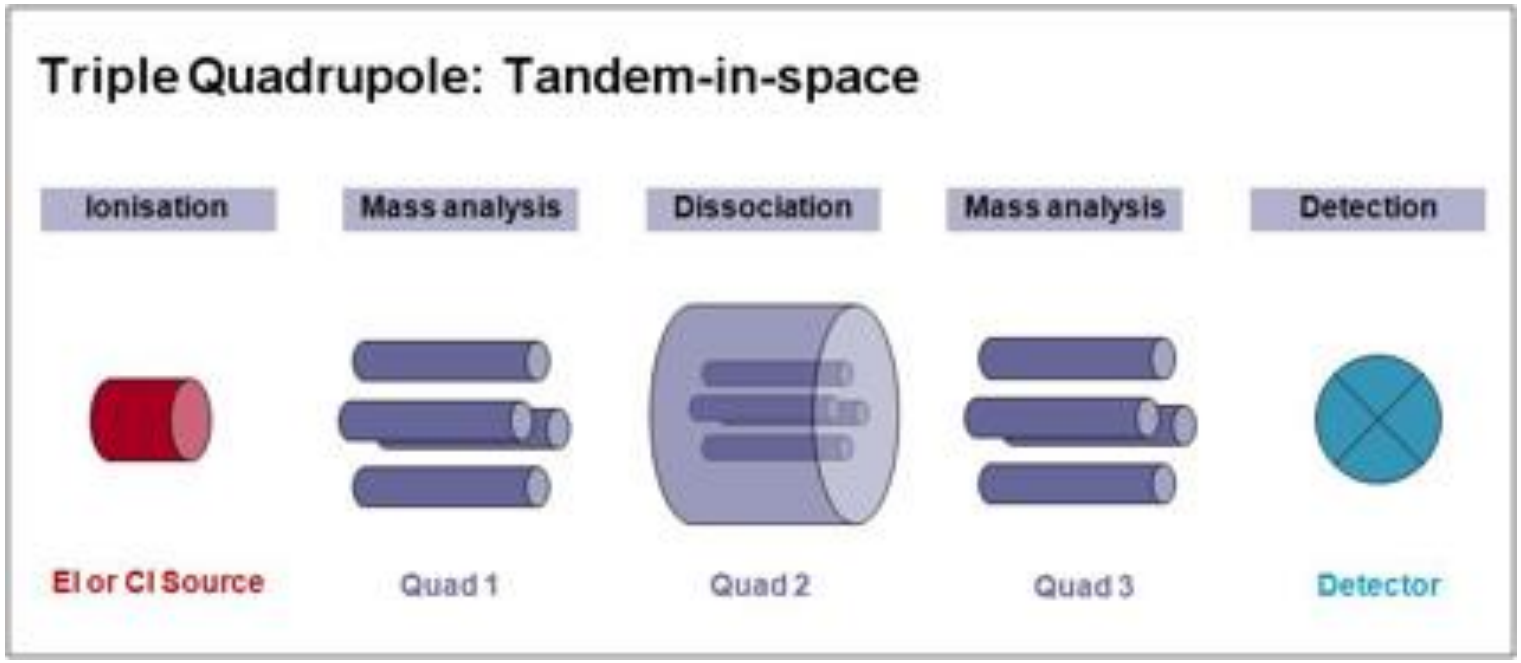
Tandem-in-Time MS/MS

Typical Tandem-in-Time MS/MS instruments include ion trap and FT-ICR MS.

Mass Spectrometry (MS)

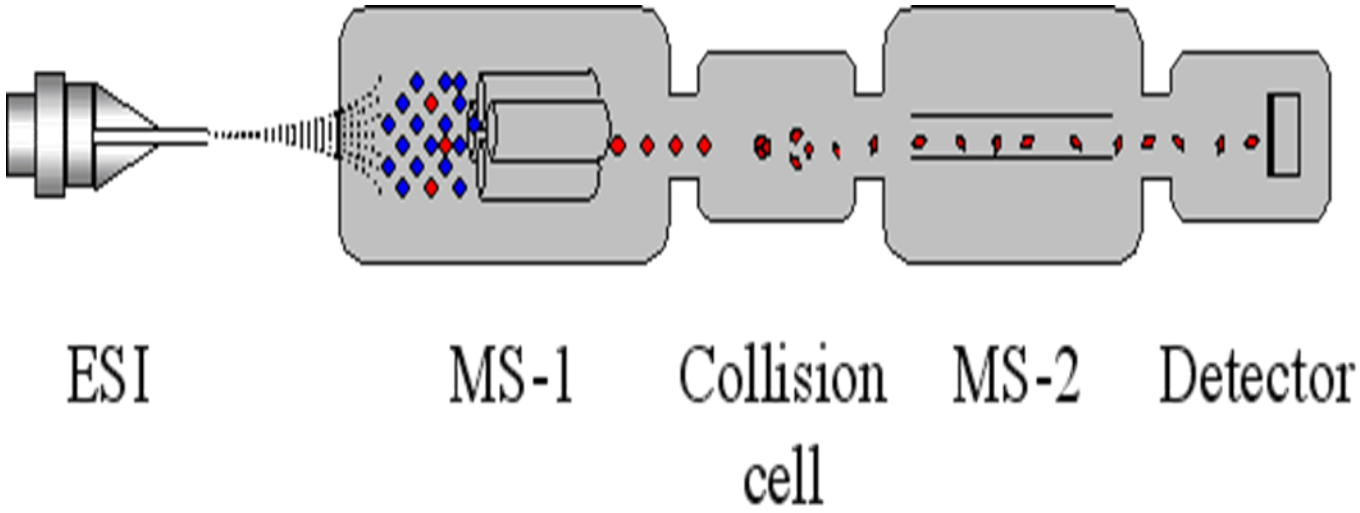
Tandem MS in space

QqQ (Triple Quadrupole)



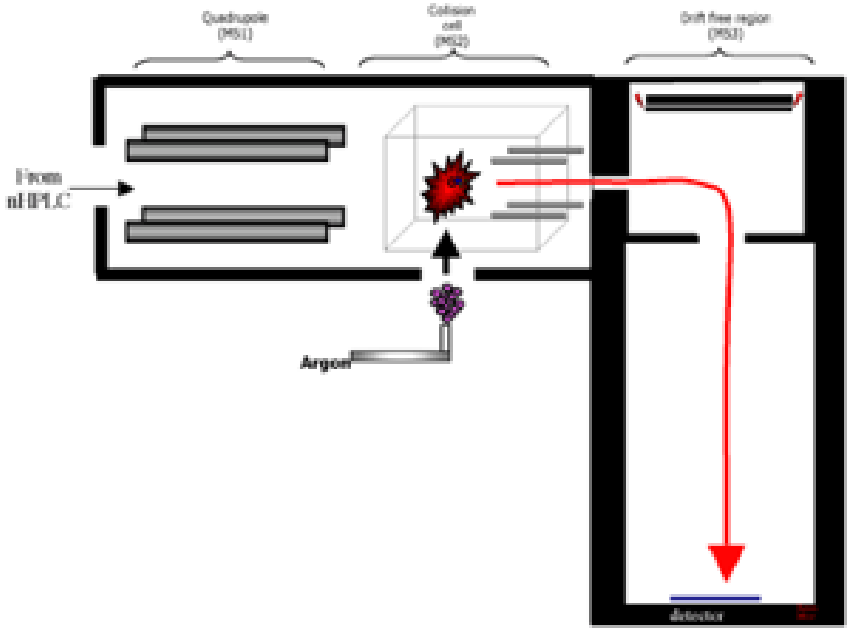
Mass Spectrometry (MS)

Three Quadrupoles (Quad 1, Quad 2, and Quad 3) are lined up in a row. Precursor ions are selected in Quad 1 and sent to Quad 2 for dissociation (fragmentation). The generated product ions are sent to Quad 3 for mass scanning.



Mass Spectrometry (MS)

QTOF (Quadrupole Time-of-flight)



In the QTOF, precursor ions are

selected in the Quadrupole and sent to the Collision Cell for fragmentation. The generated product ions are detected by time-of-flight (TOF) mass spectrometry.

Mass Spectrometry (MS)

Tandem Mass Spectrometry (MS–MS)

The two analyzers (MS-MS) can be separated by a collision cell (can be another MS) into which an inert gas (e.g. argon, xenon) is admitted to collide with the selected sample ions and bring about their fragmentation.

Tandem MS have the ability to perform multiple steps on a single sample.

The MS selects a specific ion, fragment the ion, and generate another mass spec – able to repeat the cycle several times.

Mass Spectrometry (MS)

Tandem Mass Spectrometry (MS–MS)

Collision-Induced Dissociation (CID)

To obtain structural information, analyte ions are fragmented by colliding them with neutral molecules (CID).

Voltages are applied to the analyte ions to add energy to the collisions and create more fragments.

Mass Spectrometry (MS)

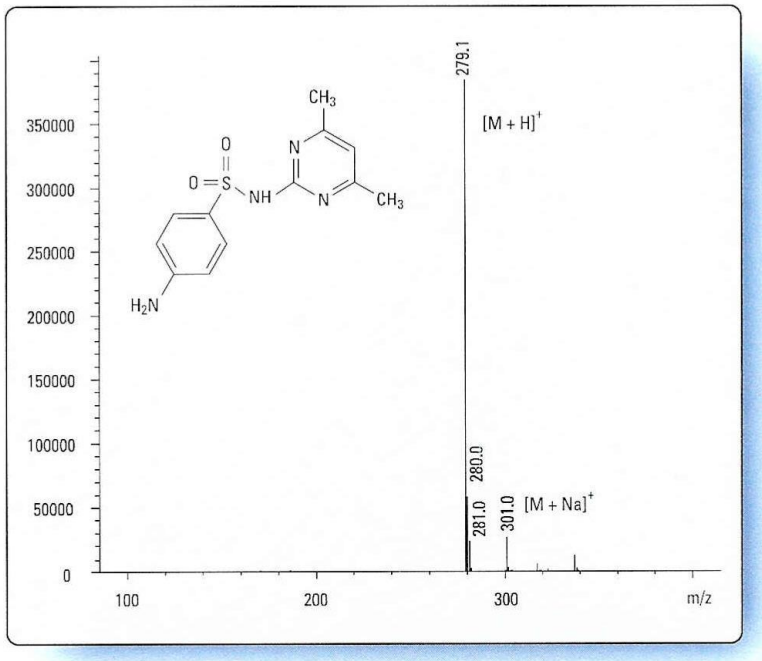


Figure 13. Mass spectrum of sulfamethazine acquired without collision-induced dissociation exhibits little fragmentation

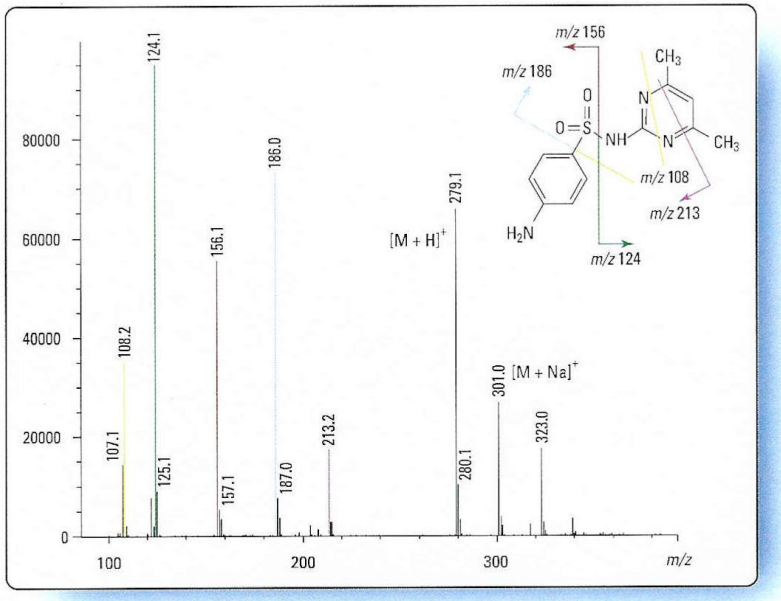


Figure 14. Mass spectrum of sulfamethazine acquired with collision-induced dissociation exhibits more fragmentation and thus more structural information

Mass Spectrometry (MS)

Tandem Mass Spectrometry (MS–MS)

The two stages of mass spectrometry are related in specific ways in order to provide the desired analytical information.

There are a large number of different collision-induced dissociation MS–MS experiments that can be carried out but the four most widely used are

(i) the product-ion scan,

(ii) the precursor-ion scan,

(iii) the constant-neutral-loss scan, and

(iv) selected decomposition monitoring.

Mass Spectrometry (MS)

Tandem Mass Spectrometry (MS–MS)

The Triple Quadrupole

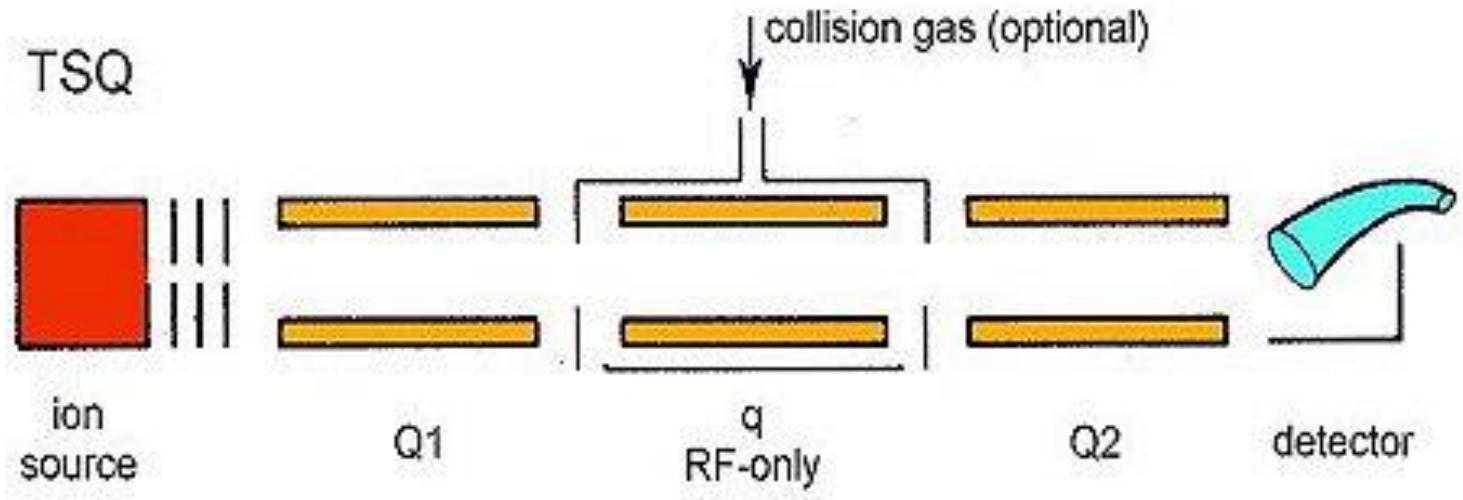
This is probably the most widely used MS–MS instrument.

The hardware, as the name suggests, consists of three sets of quadrupole rods in series.

Mass Spectrometry (MS)

The Triple Quadrupole

Triple quadrupole instruments allow MS/MS experiments to be made with ease. For this purpose, Q1 is used for mass analysis, q for fragmentation (RF-only quadrupole) and Q2 for mass analysis of ions produced within the q region.



Mass Spectrometry (MS)

Triple Quadrupole

The second set of rods is not used as a mass separation device but as a collision cell, where fragmentation of ions transmitted by the first set of quadrupole rods is carried out, and as a device for focusing any product ions into the third set of quadrupole rods.

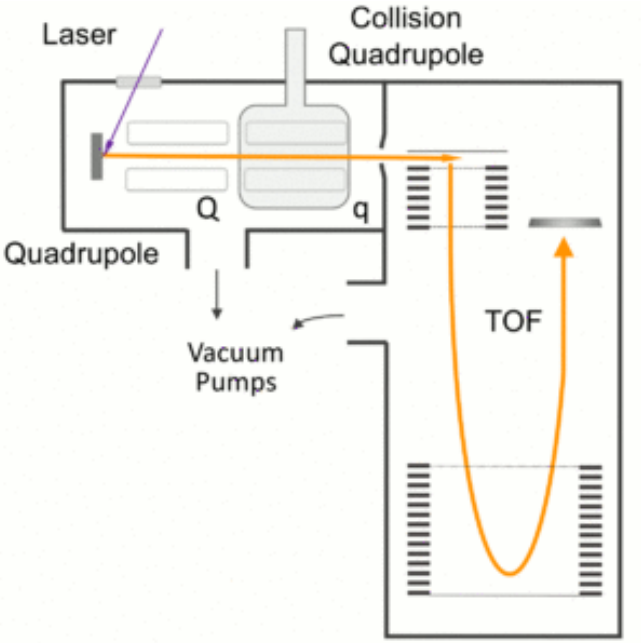
Both sets of rods may be controlled to allow the transmission of ions of a single m/z ratio or a range of m/z values to give the desired analytical information.

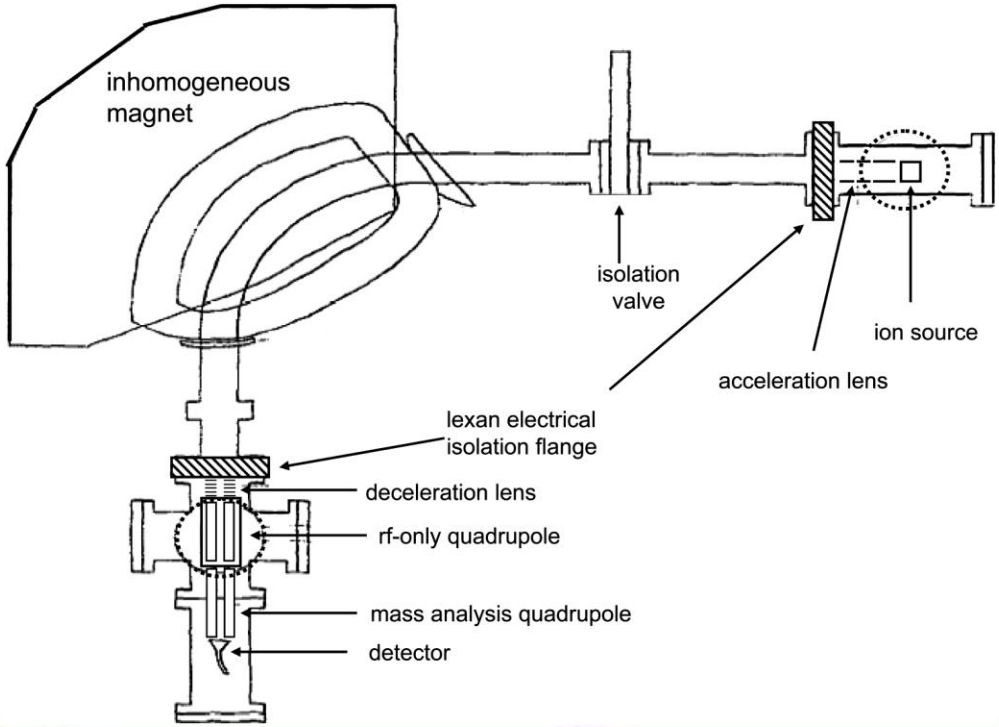
Mass Spectrometry (MS)

The Hybrid Mass Spectrometer

When a quadrupole of a triple quadrupole is replaced by a another mass analyzer, the instrument is termed a hybrid.

There are many variations.





Mass Spectrometry (MS)

The Hybrid Mass Spectrometer

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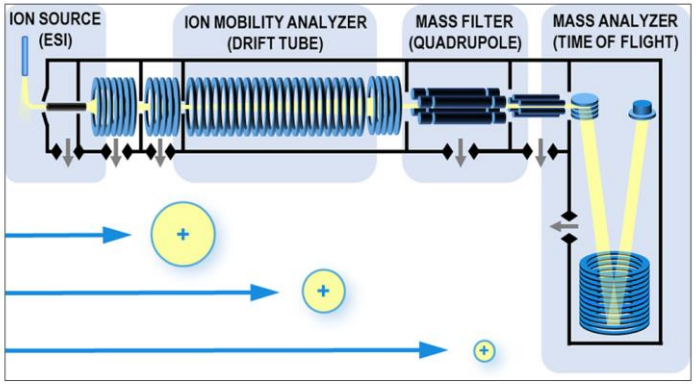
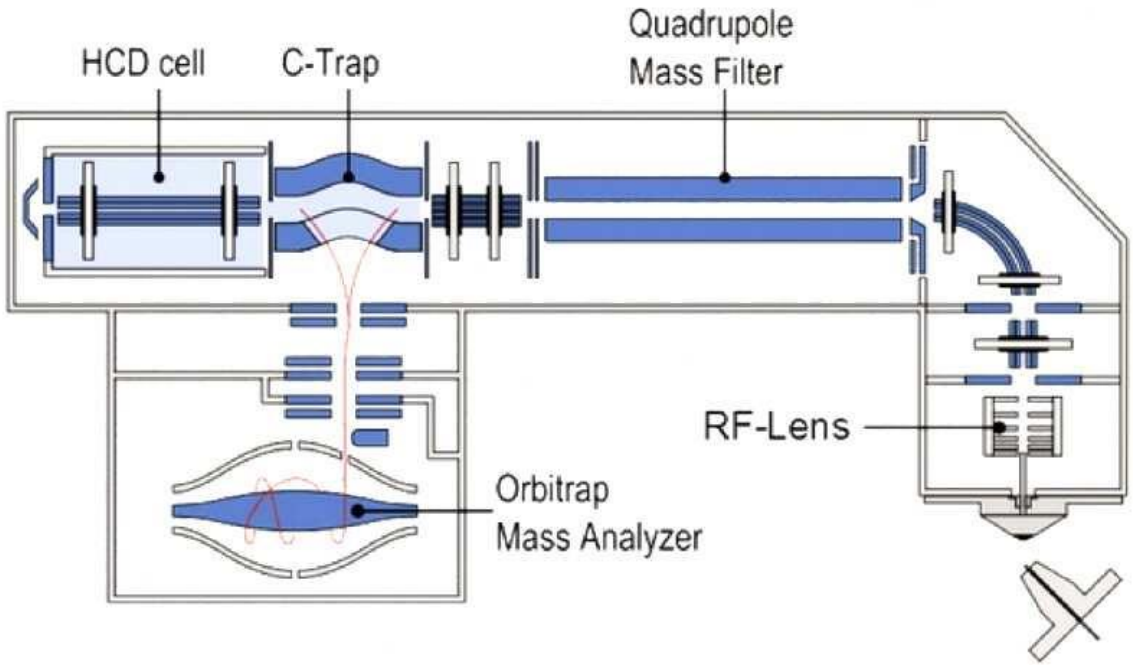
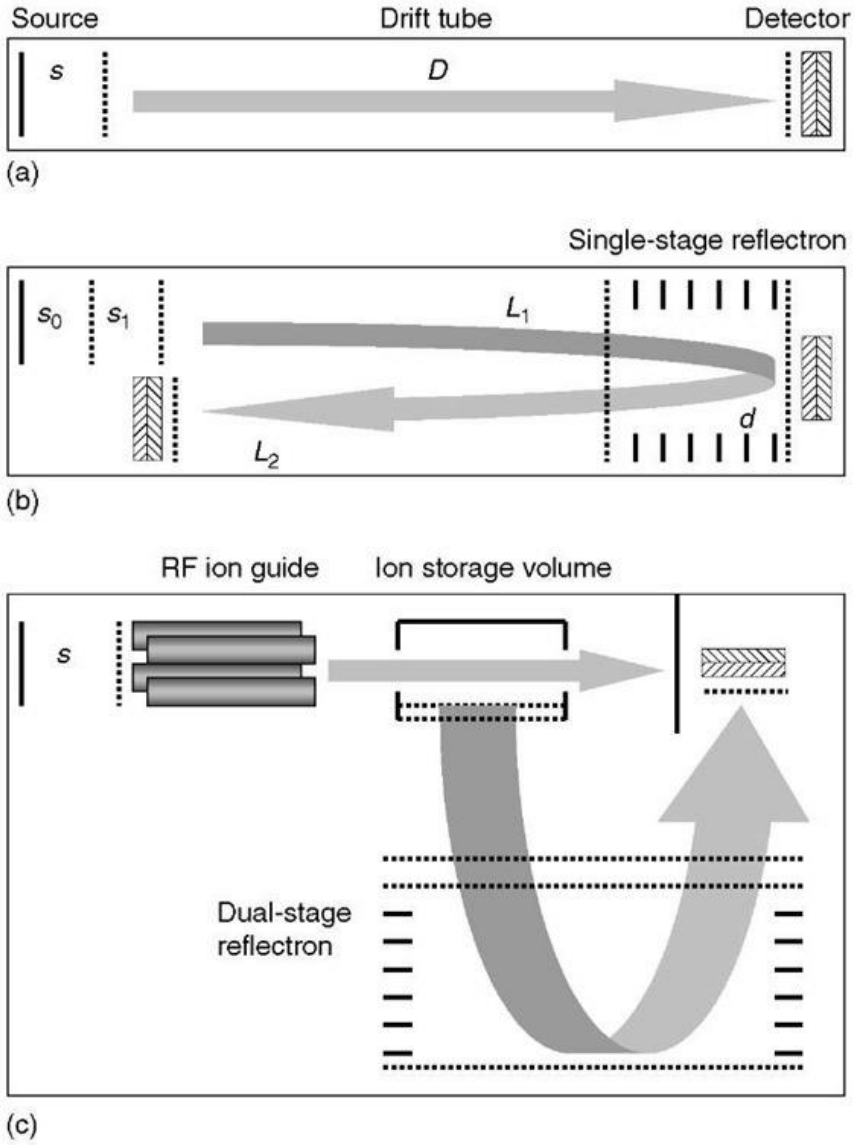




Mass Spectrometry (MS)

Basic configurations of time-of-flight mass spectrometers: (a) a simple linear TOF mass analyzer with a single-stage ionization source, (b) a reflectron TOF mass analyzer with a dual-stage ion extraction source, and (c) an orthogonal acceleration mass analyzer with a quadrupole ion guide and a dual-stage reflectron



Mass Spectrometry (MS)

Instrumentation can be simple to very complex.

Hopefully, you now have the tools to continue your knowledge of instrumental design, theory, and use.

Never stop learning. Always be curious.

Keep exploring all the different scientific instruments that are out there – and maybe invent a few of your own ☺

Assignment

- Final – Mass Spectrometry - Wednesday May 8th
- **8:00 a.m.** (No late entry – no one allowed in the room after the first student finishes)