MASS SPECTROMETRY (MS)

&

SECONDARY ION MASS SPECTROMETRY (SIMS)

Prepared and will be presented by
BUSE ATİLA
20823779

Outline

- Historical Background
- Applications of MS
- Purposes of MS
- MS Components
- MS Principles
- MS Ionization Methods
- SIMS
- MALDI
- Tandem MS
- References

Historical Background

- The work of Thomson and Aston at the Cavendish Laboratories, Cambridge University
- J.J. Thomson built MS prototype to measure m/z of electron,awarded Nobel Prize in 1906
- Design to measure mass of elements
- Aston awarded Nobel Prize in 1922
- 1948-52 Time-of-flight (TOF)
- 1955 Ouadrupole ion filters –W.Paul (1989 Nobel Prize)
- 1968-Tandem Mass Spectrometry

An analytical technique ;

- 1) It's objective to ionize a gas phase molecule and then analyze the masses of it's produced ions
- Ions ,an atom or a group of atoms, electrically charged.They lose or gain electrons,thus they are negatively or positively charged.
- 2) The produced ions are separated to according to their mass-to-charge ratio (m/z) and then the relative abundance of each ionic species present is recorded (measured).

- 3)Quantitative analysis of atoms and molecules because of m/z ratio.
- **MS Applications**
- In industry and academia. Major mass spectrometric applications:
- **Biotechnology:** The analysis of proteins, peptides, oligonucleotides
- **Pharmaceutical:** Drug discovery, combinatorial chemistry, pharmacokinetics, drug metabolism
- **Clinical:** Newborn screening, hemoglobin analysis, drug testing
- **Environmental:** PAHs, PCBs, water quality, food contamination
- **Geological:** Oil composition

For what purposes MS is used?

- For measuring the molecular mass of a sample
- For identification of the unknown compounds
- little as 10^{-12}g, 10^{-15} moles ; compounds identified at very low concentrations (one part in 10^{12}) in chemically complex mixtures
- For quantification of known compounds
- Used to discover the number of isotopes ,determine the relative abundance of the isotopes and measure of their exact masses

For what purposes MS is used?

- Within an accuracy of 0.01% of total weight of sample and within 5 ppm for small organic molecules
- helps to determine the chemical and structural information about molecules
- Fragment sample & analyse products
- Uses a machine called Mass Spectrometer.

MS COMPONENTS

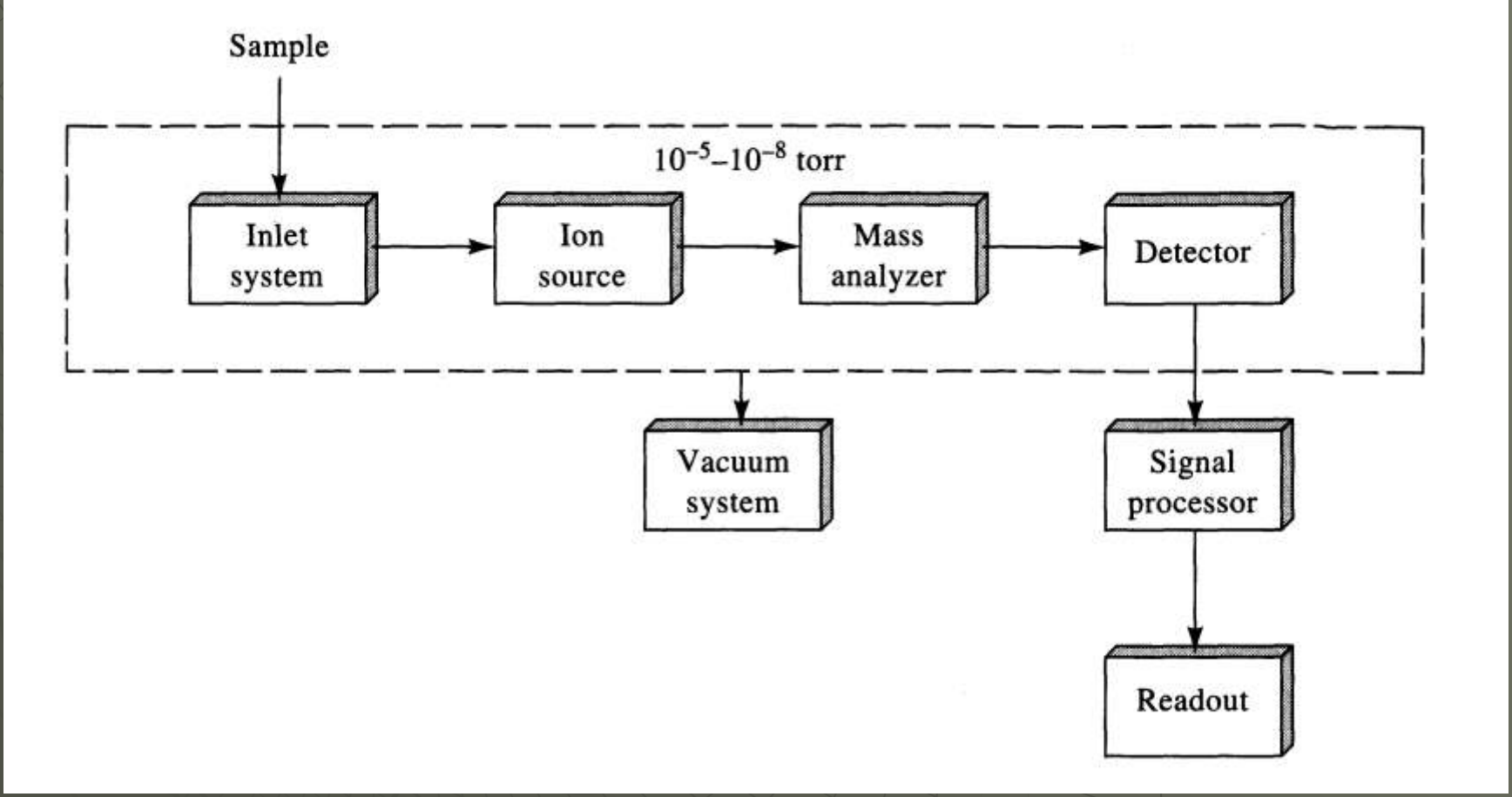
- Depend on the used ionization method, the type and the complexity of the sample

- 3 major components ;

1. An ion source,
2. A mass analyzer and
3. A detector

- A sample introduction system (inlet system)

Schematic Diagram of a MS



1)The ion source;

- produces gaseous ions from the sample;
- Samples easier to manipulate if ionised because ions are easier to manipulate than neutral molecules
- a small sample of compound is ionized especially cations are produced by lossing electrons

2) The mass analyzer ;

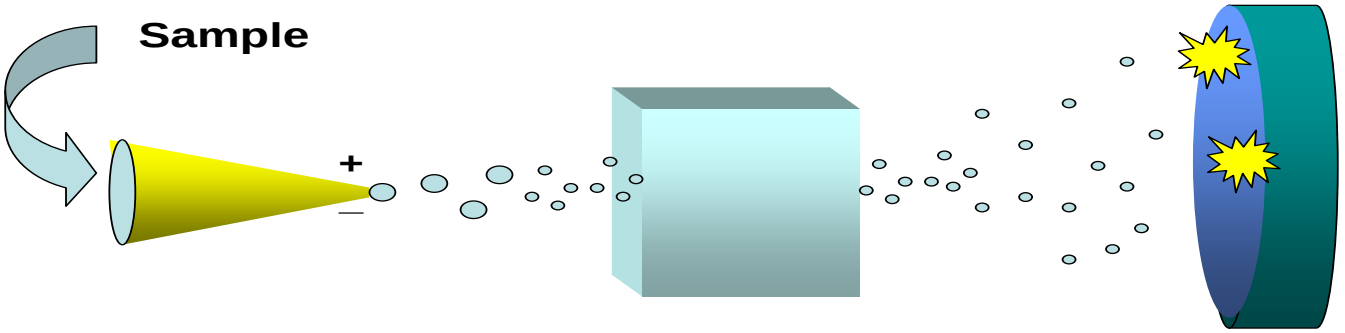
- separates ions according to m/z
- These ions are extracted into the analyser region of the mass spectrometer

3) The detector

4) The sample introduction;

- **by introduction into the ion source**
- **by ejecting of charged molecular species from a solid surface or solution**

Mass Spec Principles

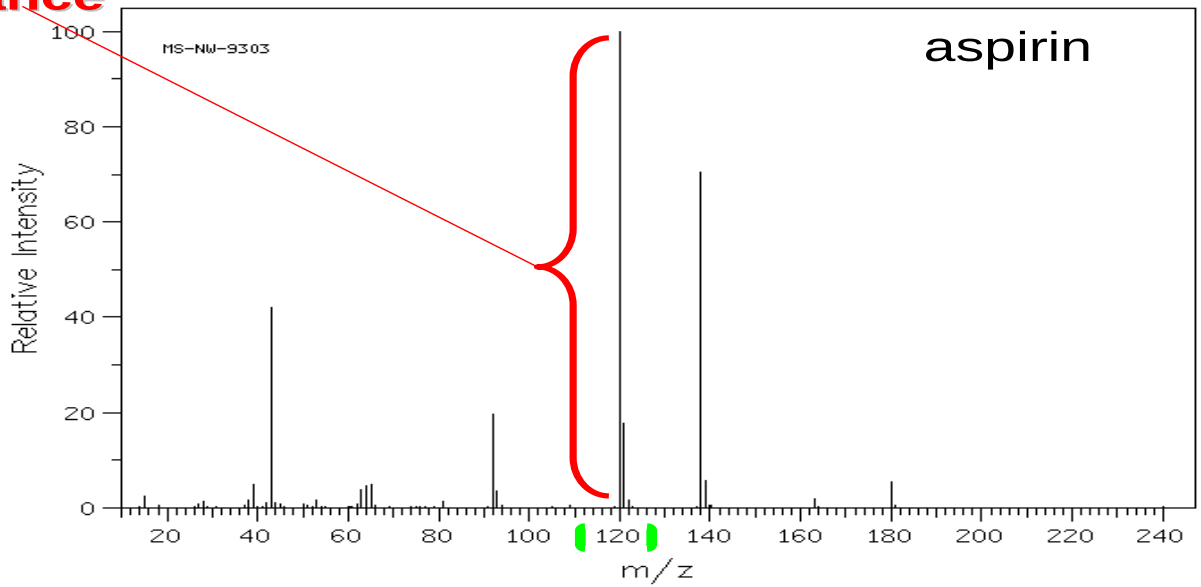


Why operating in a high-vacuum system?

- Because of formating and manipulating gas-phase ions
- Ions are very reactive and short-lived
- Atmospheric pressure, 1 atm=760 Torr.Ions are handled in the pressure of 10^{-5} to 10^{-8} torr
- Allow unhindered movements of ions
- Mechanical and diffusion pump

Typical Mass Spectrum

Relative Abundance



120 m/z-for singly charged ion this is the mass

ACCURACY & RESOLUTION

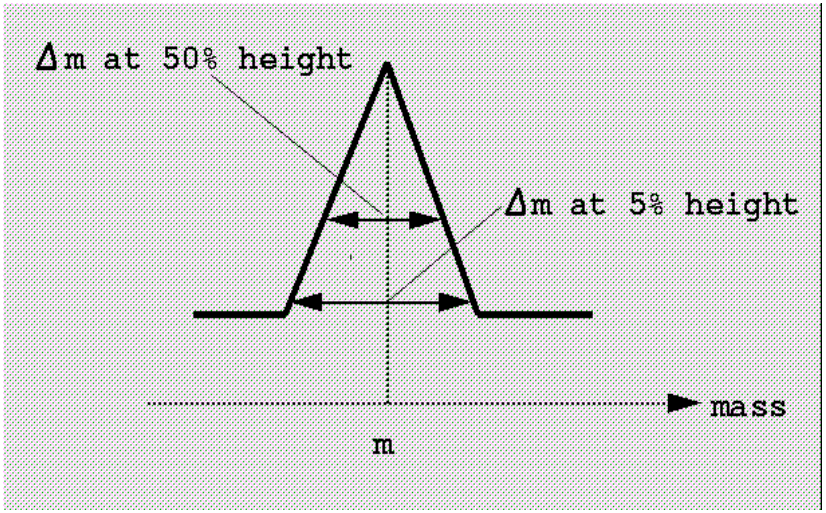
The ability of a spectrometer to separate two adjacent peaks in a spectrum

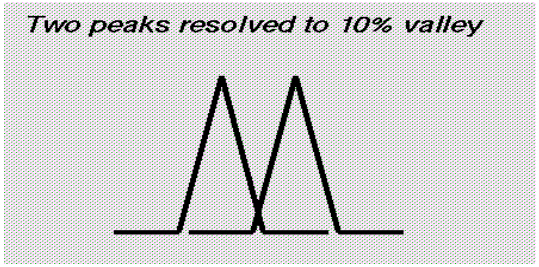
- For large samples such as **biomolecules,** an accuracy of **0.01%** of the total molecular mass of the sample
- For small **organic molecules** , an accuracy of **5 ppm** or less
- For the suitable instrument
- Showed by width of the peak
- The increase in resolution or resolving power, the more qualified instrument ,mass accuracy increases

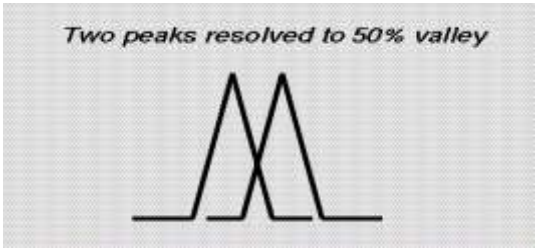
$$R = \frac{M}{\Delta M}$$

- M=the mass number
- ΔM= the difference between two masses

Resolution in MS







Mass Spectrometry Ionization Methods

- Chemical ionization (CI)
- Plasma and glow discharge
- Electron impact (EI)
- Electrospray ionization (ESI)
- Fast-atom bombardment (FAB)
- Field ionization
- Laser ionization (LIMS)
- Matrix-assisted laser desorption ionization (MALDI)
- Plasma-desorption ionization (PD)
- Resonance ionization (RIMS)
- Secondary ionization (SIMS)
- Spark source
- Thermal ionization (TIMS)

Electron Impact (EI)

- The original mass spectrometry (MS) ionization method
- The most widely used
- Used for ionization and fragmentation the sample molecules before mass analysis
- Firstly the sample is vaporized and then enters into the ion source
- A beam of electrons help the sample be impacted with sufficient energy to ionize the molecule
- $M(g) + e^- \rightarrow M^+(g) + 2e^-$
- M^+ is molecular ion molecular weight analytical information
- $M_1^+ \rightarrow M_2 + + M_n$
- M_1 is the molecular ion (odd)

The fragmentation process

- Structural information about the molecule
- Fragment ions= odd electron or even electron.
  - primary structure,
  - electron energy 70 eV energy
- Ion source temperature
- **The molecular ion is a radical which is** not very stable and tend to fragment.
- EI mass spectra intense fragment ion peaks and much less intense molecular ion peak
- When the molecular ion peak is not observed in the mass spectrum,CI can be used in order to get molecular ion information
- EI ionization requires that the molecules are vaporized before ionization.
- Volatile under the conditions of the ion source
- Sample introduction to the EI source by

1. a gas chromatography device
2. a solids probe device.

Fast-Atom Bombardment (FAB)

- Ion production in a mass spectrometer from nonvolatile or thermally fragile organic molecules by bombarding the compound
- A technique for the analysis of protein sequence and structure to various organometallic systems
- A high energy beam of atoms or ions (Cs or Xe) vapourizes, fragments and ionizes a protein solution
- Flow FAB is a variant that analyses the continuous liquid stream from a HPLC or capillary electrophoresis separation

Electrospray Ionization (ESI)

- Soft ionization technique, desorption ionization method
- Solid or liquid samples, nonvolatile or thermally unstable.
- Proteins, peptides, and other biological macromolecules
- No fragmentation of the macromolecules into smaller charged particles; turning the macromolecule being ionized into small droplets
- Large mass molecules are detected in the small mass range of the instrument
- As the m/z value increases, the number of protons attached to the molecular ion decreases
- When mixtures are used as the analyte, this technique is out of use
- Purification methods ;HPLC , Capillary Electrophoresis , and Liquid-Solid Column Chromatography

- Advantages:
- samples with large masses can be handled
- analyzing biological samples that are defined by non-covalent interactions because of soft ionization method
- Disadvantages:
- cannot analyze mixtures very well
- the apparatus becomes contaminated quickly and hard to clean
- the multiple charges that are attached to the molecular ions can make for confusing spectral data.

Secondary Ionization (SIMS)

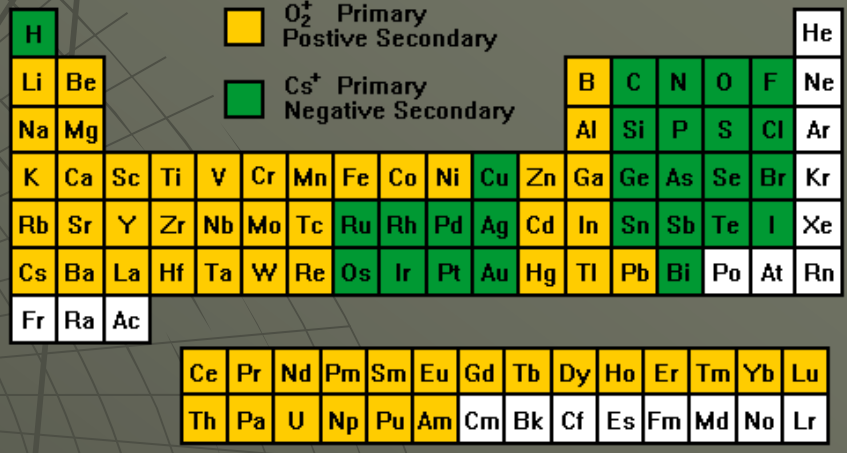
- Organic and inorganic analyses ; spectrometers quite different
- Surface analysis technique used to characterize the surface and sub-surface region of materials.
- Energetic primary particles (electrons, ions, neutrals or photons)
- Cause bombardment on a solid surface A primary ion beam; such as $^{3}He^{+}$,$^{16}O^{+}$, or $^{40}Ar^{+}$
- Thus mass spectrometer of ionised particles which are emitted occur

The Sputtering Process(bombardment)

- Binary collisions (elastic and inelastic) of primary ions with single target atoms
- Oxygen increases positive ions and cesium increases negative ion
- Metal-oxygen bonds in an oxygen rich zone help oxygen enhance
- Bonds break in the ion emission process, oxygen negatively charged ;metal positively charged

- Oxygen beam sputtering increases the concentration of oxygen in the surface layer.
- More secondary electrons are excited over the surface potential barrier.
- Increased availability of electrons leads to increased negative ion formation.

- The variability in ionization efficiencies for different elements

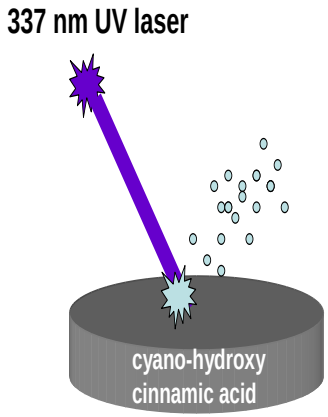


- ***Advantages of SIMS***
- has a very low detection limit (ppm to ppt)
- detection ability for all elements
- material can be continually sputtered from a surface to determine analyte concentrations as a function of distance from the original surface (depth profiling).
- ***Disadvantages of SIMS***
- a very great range of ionization rates for different elements
- matrix effects; the rates vary depending on the other species present
- response factors are different for either a positive and a negative beam; difficult quantification

Matrix-Assisted Laser Desorption Ionization (MALDI)

- A laser-based soft ionization method
- Important for protein analysis.
- Ionization occurs with bombarding the sample with laser light
- The sample is embedded in a chemical matrix (UV absorbant)
- The matrix makes the production of intact gas-phase ions from large, nonvolatile, and thermally decomposed compounds such as proteins, oligonucleotides, synthetic polymers easy
- The matrix absorbs the laser light energy thus small amount is vaporized

Matrix-Assisted Laser Desorption Ionization



MALDI

- The MALDI matrix must meet a number of requirements simultaneously:
- be able to embed and isolate analytes (e.g., by co-crystallization)
- be soluble in solvents compatible with analyte
- be vacuum stable
- absorb the laser wavelength
- promote analyte ionization

Mass Analyzers

- Separation ions depending on m/z ratio
- Magnetic-sector analyzer
- Quadrupole analyzer
- Time-of-flight analyzer
- FT-Ion cyclotron analyzer
- Ion-trap analyzer

Time-of-Flight Analyzer

- Consists of 3 parts: an ion-accelerating region, a flight tube, and a detector
- Used with the MALDI technique for analysis of large biomolecules
- All ions are created in the ion-accelerating region
- Ions are pulsed into the flight tube in short, well defined packets. all ions have same kinetic energy ,same potential difference and a constant homogeneous electrostatic field subjection during acceleration .
- Thus having same kinetic energy leads different velocities depending on their masses
- Masses affect their arrival time at the detector and within order of increasing mass they reach the detector
- The differences of flight times are, the basis for resolving ions of different *m/z* and the mass resolution depends on the flight time differences
- Its insufficient mass resolution,because flight time variations of ions of the same *m/z*

ADVANTAGES:

- Unlimited mass range
- Ideal for Pulsed ionization or spatially confined
- For each ionization complete mass spectrum
- High transmission
- Valid for extremely small sample amounts
- Relatively low cost
- Fast mass scanning

DISADVANTAGES:

- The pulse is not felt by all ions to the same intensity and so a kinetic energy distribution for each discrete *m/z* exists. This lowers the resolution by creating a time-of-flight distribution for each *m/z*

Quadrupole Analyzer

- four parallel metal rods
- an ion source,
- ion optics ,
- the quadrupole filter itself with control voltage supplies,
- an exit aperture,
- an ion detector,
- detection electronics,
- and a high-vacuum system.
- (U+Vcos(wt)) = the potential of two opposite rods
- - (U+Vcos(wt)) =the potential of other rods
- U = a dc voltage and Vcos(wt) =an ac voltage
- ions travel down the flight path which is centered between the four rods, and because of the applied voltages the trajectory of ions is affected.
- This filter is selective of ions depending on their certain m/z and Vac/Vdc ratios:some of them pass and the others are thrown out.
- Voltages on the rod vary and mass spectrum is obtained
- There are two methods: varying w and holding U and V constant, or varying U and V (U/V) fixed for a constant w.

Ion-Trap Analyzer

- Three electrodes with hyperbolic surfaces to trap ions in a small volume
- A central ring electrode two adjacent endcap electrodes
- After changing the electrode voltages for ejection of ions from the trap , a mass spectrum is obtained.
- ADVANTAGES
- Compactness
- The ability to trap and accumulate ions to increase the signal-to-noise ratio of a measurement.
- High sensitivity
- Available for tandem mass spectrometry
- İon/ molecule reactions can be studied for mass-selected ions
- High resolution
- DISADVANTAGES
- Mass measurement accuracy is relatively poor

FT-Ion Cyclotron Analyzer

- A high-frequency mass spectrometer
- Because of a high-frequency field., the ions absorb maximum energy with a selected m/z ratio
- The cyclotron resonance condition is satisfied by ions after max energy is gained
- And then separation from ions of different m/z
- Cyclotron motion of ions (having different m/z ratios) in a constant B
- This motion is excited simultaneously by a pulse of a RF electric field applied perpendicularly to B
- Has a higher mass resolution than any other mass analyzer available
- Superconducting magnets are used in FT-ICR MS

Magnetic-Sector Analyzer

- 'B' sector and 'E' sector
- the path of a *moving* charged particle (ion) is curved in the sector by a magnetic field.
- A *stationary*, charged, non-magnetic particle is not attracted or repelled by a magnetic field.
- The ions different speed, but have the same kinetic energy
- They enter the magnetic sector through the source slit where they are deflected according to the left-hand rule.
- Detection of ions that have different m/z consecutively
- Constant magnetic field, kinetic energy sweep larger B : larger m/z
- constant kinetic energy, magnetic field sweep larger V : smaller m/z
- Higher-mass ions are deflected less than lower-mass ions
- high-resolution capabilities.
- Can not scan the field of a superconducting magnet, it has a fixed field strength

MS Detectors

1. **Photomultiplier**
2. **Microchannel plate**
3. **Electron multiplier**

Tandem Mass Spectrometry

- The simplest MS /MS method
- analysis according to the mass/charge ratio.
- Two mass analyzers or filters between them a collision cell filled with Argon or Xenon
- The sample sorted and weighed in the first mass spectrometer, then broken into pieces in the collision cell, and a piece or pieces sorted and weighed in the second mass spectrometer
- Separation and identification of compounds in complex molecules
- Cause fragmentation and mass analyze of fragment ions
- in newborn screening to detect molecules such as amino acids and fatty acids.

REFERENCES

- http://www.forumsci.co.il/HPLC/usp-ms-2spaltet.pdf
- http://www.cem.msu.edu/~cem333/Week18.pdf
- http://www.bioacademy.gr/bioinformatics/courses/Helsinki%207_11May07_projects/Introduction%20to%20Mass%20Spectrometry.pdf
- http://www.files.chem.vt.edu/chem-ed/ms/ms-intro.html
- http://www.astbury.leeds.ac.uk/facil/MStut/mstutorial.htm
- http://theory.caltech.edu/~politzer/supplements/mass-spectroscopy.pdf
- http://spectroscopyonline.findanalytichem.com/spectroscopy/article/articleDetail.jsp?id=197393
- http://www.jic.ac.uk/services/proteomics/tof.htm
- http://www.rmjordan.com/Resources/Tutorial.pdf
- http://www.chm.bris.ac.uk/ms/theory/tof-massspec.html
- http://www.files.chem.vt.edu/chem-ed/ms/quadrupo.html
- http://www.chemistry.adelaide.edu.au/external/soc-rel/content/ion-trap.htm
- http://www.currentseparations.com/issues/16-3/cs16-3c.pdf
- http://old.iupac.org/publications/analytical_compendium/Cha12sec2.pdf

REFERENCES

- http://chemistry.clemson.edu/chemdocs/marcusgroup/software/CONCEPT/SMA/SMA.HTM
- http://www.chm.bris.ac.uk/ms/theory/sector-massspec.html
- http://www-chem.harvard.edu/mass/tutorials/magnets.html
- http://www.chem.harvard.edu/mass/tutorials/ei.html
- http://www.public.iastate.edu/~kamel/ei.html
- http://old.iupac.org/goldbook/F02328.pdf
- http://www.genscript.com/product_003/molecular_biology_glossary/id/1071/category/glossary/fast_atom_bombardment_mass_spectrometry_FAB_MS_.html
- http://chemwiki.ucdavis.edu/Analytical_Chemistry/Instrumental_Analysis/Mass_Spectrometry/Electrospray_Ionization_Mass_Spectrometry
- http://www.sigmaaldrich.com/analytical-chromatography/spectroscopy/maldi-mass.html
- http://www.tau.ac.il/lifesci/units/proteomics/voyager.html
- http://www.eaglabs.com/training/tutorials/sims_theory_tutorial/beameff.php
- http://books.google.com.tr/books?id=8C7pXhnqje4C&pg=PA80&lpg=PA80&dq=Advantages+of+SIMS&source=bl&ots=tpAPhzLsJ7&sig=XQf1RwQCE0wXpvCVvtk6cRCu_eI&hl=tr&ei=VievTZ3tBsXNswbF0KzhDA&sa=X&oi=book_result&ct=result&resnum=10&ved=0CG4Q6AEwCQ#v=onepage&q=Advantages%20of%20SIMS&f=false
- http://www.medterms.com/script/main/art.asp?articlekey=25329
- http://www.colorado.edu/chemistry/chem5181/MS3_Ionization_II_2006.pdf
- http://ittpowersolutions.com/commercial-products/ion-detectors.asp
- http://www.files.chem.vt.edu/chem-ed/optics/detector/pmt.html
- http://imagine.gsfc.nasa.gov/docs/science/how_l2/microchannels.html
- http://www.sge.com/products/electron-multipliers