Mass Analyzers

Árpád Somogyi

Campus Chemical Instrument Center
Ohio State University

Mass Spec and Proteomics Workshop
July 25, 2022
Sample Preparation

Sample Introduction
- Direct probe/infusion
- GC
- HPLC

Vacuum System
- Rough,
- Turbomolecular,
- and
- Cryo pumps

Ionization Source
- Electron impact (EI)
- Chemical ionization (CI)
- Atmospheric pressure (API)
- Electrospray (ESI)
- Matrix assisted laser
- Desorption/ionization (MALDI)
- Surface enhanced LDI (SELDI)
- Fast atom bombardment (FAB)

Mass Analyzer
- Electrostatic (ESA)
- Magnet (B)
- Time-of-flight (TOF)
- Quadrupole (Q)
- Ion Traps (2D & 3D IT)
- Ion-Cyclotron Resonance (ICR)
- Orbitrap (OT)

Detector
- Electron Multiplier
- Photomultiplier
- Faraday cap
- Array Detectors
- Multichannel plate

Computer
Want to do MS or MS/MS? Need a Mass Spectrometer

Ionization source → all ions → Mass analyzer → sorted ions → Detector → Data system
Ion movement

- Biased metal plates (electrodes, lenses) used to move ions between ion source and analyzers

- Electrodes and physical slits used to shape and restrict ion beam

- Good sensitivity is dependent on good ion transmittance efficiency in this area
Ion detection

- Ions can be detected efficiently with high amplification by accelerating them into surfaces that eject electrons
Detectors

Principle of the (Discrete) Electron Multiplier

Continuous Dynode Electron Multiplier

Microchannel Plate Detector

w/ TOF
Image Current Detectors

w/ FTICR

RF-excitation

Ion Detection Plates

B

image current

w/ Orbitrap

D

D
Ubiquitin multiply charged states no selection

Ubiquitin +11 charged state Q selection

image current
Ion energy

- Kinetic energy of ions defined by

\[ E = z e V = q V = \frac{1}{2} m v^2 \]

- **E** = kinetic energy
- **m** = mass
- **v** = velocity
- **e** = electronic charge \((1.60217 \times 10^{-19} \text{ C})\)
- **z** = nominal charge
- **V** = accelerating voltage
Learning Check

Consider two electrodes,

one at 1000 V and one at ground (0 V)

+ ion will travel with kinetic energy of ____________
Question: Consider an ion source block and an extraction lens. How would you bias the block and lens if you want the ions to be accelerated by

a) 8000 eV (appropriate for magnetic sector)

b) 5 eV (appropriate for entering a quadrupole)

c) 20,000 eV (appropriate for entering a TOF)
Mass Resolution

• Mass spectrometers separate ions with a defined *resolution/resolving power*

• *Resolving power* - the ability of a mass spectrometer to separate ions with different mass to charge (m/z) ratios.
Resolution defined at 10% valley

\[ R = \frac{M}{\Delta M} \]

\[ R = \frac{1000}{1} = 1000 \]
Example of ultrahigh resolution in an FTICR
“Kaba” meteorite powder
Laser desorption/ionization (LDI)
Bruker 15 T FT-ICR

2e⁻ difference between positively and negatively charged species detected
General about mass spectrometers

• **Common features**
  – Accelerated charged species (ions) interact with/can be controlled by
    • Electrostatic field (ESA, OT)
    • Magnetic field (B, ICR)
    • Electromagnetic (rf) fields (Q, IT, LT)
  – Or ions just fly (TOF)
For each of the following applications, choose the most appropriate mass analyzer from the following list.

- orbitrap (OT)
- quadrupole (Q)
- time-of-flight (TOF)
- FTICR

<table>
<thead>
<tr>
<th>Analyzer</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>synthetic organic chemist wants exact mass of compound</td>
<td>________ synthetic organic chemist wants exact mass of compound</td>
</tr>
<tr>
<td>biochemist wants protein molecular weight of relatively large protein (MW 300,000)</td>
<td>________ biochemist wants protein molecular weight of relatively large protein (MW 300,000)</td>
</tr>
<tr>
<td>EPA (Environmental Protection Agency) wants confirmation of benzene in extracts from 3000 soil samples</td>
<td>________ EPA (Environmental Protection Agency) wants confirmation of benzene in extracts from 3000 soil samples</td>
</tr>
<tr>
<td>Petroleum chemist wants to confirm the presence of 55 unique compounds at one nominal mass/charge value in a mass spectrum</td>
<td>________ Petroleum chemist wants to confirm the presence of 55 unique compounds at one nominal mass/charge value in a mass spectrum</td>
</tr>
</tbody>
</table>
Desirable mass analyzers characteristics
Desirable mass analyzers characteristics

1) They should sort ions by $m/z$

2) They should have good transmission (improves sensitivity)

3) They should have appropriate resolution (helps selectivity)

4) They should have appropriate upper $m/z$ limit

5) They should be compatible with source output (pulsed or continuous)
Mass spectrometers record \( m/z \) values

\( m/z \) values are determined by actually measuring different physical parameters

**Type of analyzer**
- electric sector
- magnetic sector
- quadrupole, ion trap
- time-of-flight
- FT-ion cyclotron resonance

**Physical parameter used as basis for separation**
- kinetic energy/z
- momentum/z
- \( m/z \)
- flight time
- \( m/z \) (resonance frequencies)
Types of Mass Analyzers

- Magnetic (B) and/or Electrostatic (E) (HISTORIC/OLDEST)
- Time-of-flight (TOF)
- Quadrupole (Q)
- Quadrupole Ion Trap (IT)
- Linear Ion Trap (LT)
- Orbitrap
- Fourier Transform-Ion Cyclotron Resonance (ICR)

Performance Advantages / Disadvantages / $$$
Notice: accelerating voltages vary with analyzer (has consequences for MS/MS)

• High voltage (keV energy range)
  – magnet (B)
  – electrostatic (E)
  – time-of-flight (TOF)

• Low voltage (eV energy range)
  – quadrupole (Q)
  – ion trap (IT, LT)
  – ion cyclotron resonance (ICR)
  – orbitrap
MS and MS/MS revisited

MS:

Ionization

Analysis

MS/MS:

Ionization

Selection

Activation

Analysis

Collide with target to produce fragments
Simulation of Two-Step Process

Current popular MS/MS arrangements

Tandem in Space

QqQ
Q Trap
Q TOF
TOF TOF
LTQ-Orbi (& QExactive)

Tandem in Time

Ion Trap (2 or 3 D)
FT-ICR (FT)
Decision factors when choosing a mass spectrometer

- Speed
- Resolution
- Sensitivity
- Dynamic range
- Cost
Disclaimers:

We are not intentionally favoring any single manufacturer. We are more familiar with the operation of instruments we use.

Some of our slides involve simplifications. Listeners could likely find an exception for almost any statement we make.
Time of Flight

KE = zeV = \( \frac{1}{2}mv^2 \)

\( v = \frac{D}{t} \)

\( \frac{1}{2}m(D/t)^2 = zeV \)

\( t = \left( \frac{m}{2zeV} \right)^{1/2}D \)

m = mass
V = velocity
D = distance of flight
t = time of flight
KE = kinetic energy
e = charge
How does the ion generation step in TOF influence $m/z$ analysis?

Consider MALDI

Analyte ion may have (1) Kinetic Energy distribution or (2) Spatial distribution
How will KE spread influence the spectrum?

Ions of same m/z

Has slightly greater KE

Effect is broad peaks
How will KE spread influence the spectrum?

Solution to peak broadening caused by kinetic energy spread:

Reflectron (ion mirror)

Series of ring electrodes, typically with linear voltage gradient
Time-of-Flight Reflectron

- Increased resolution by compensating for KE spread from the source

http://www.jic.bbsrc.ac.uk/services/proteomics/tof.htm
Reflectron TOF

KE = \(\frac{1}{2} mv^2\)

Ion Source (MALDI)

Mass Analyzer (TOF)

Detector

Reflectron

High resolution
KE = \( \frac{1}{2} mv^2 \)
We talked about how to deal with kinetic energy spread.

How do we deal with ions formed at different locations in the source (spatial distribution)?

**Figure 2.4** Two ions formed in different locations with respect to the backing plate, and the effects of spatial distributions on the mass spectrum.
Low mass (below 40 k Da)
High resolution
Continuous TOF
Resolution = 700 (FWHM)

Delayed Extraction
Resolution = 6000 (FWHM)
Recent TOF designs: improved resolution, better sensitivity and mass accuracy (Ultraflex III MALDI TOF-TOF)

Resolution: 25,000
S/N: 46
Typical TOF Specs

- \( m/z \) Range: unlimited u
- Resolution: \( \sim 20,000 \) (Reflectron)
- Mass Accuracy: \( \sim 3-10 \) ppm (300-4,000 u)
- Scan Speed: \( 10^6 \) u/s
- Vacuum: \( 10^{-7} \) Torr

- Quantification: low - medium
- Positive and Negative Ions
- Variations: Linear, Reflectron,
  - Tandem: w/ quads, TOFs and/or sectors
<table>
<thead>
<tr>
<th></th>
<th>TOF</th>
<th>Quad</th>
<th>3D Trap</th>
<th>Linear Trap</th>
<th>FT-ICR</th>
<th>Orbitrap</th>
</tr>
</thead>
<tbody>
<tr>
<td>mass range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resolution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>exact m/z</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all ion detection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ion storage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>speed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dynamic range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>quantification</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS/MS types</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sample introduction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>simplicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cost/performance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
MALDI TOF-TOF fragmentation spectrum of a sodiated polymer

3-OEB, m=1-3 (164, 44)
Copolymer of 2-hydroxybenzoic acid and ethylene carbonate
# Advantages and Disadvantages

<table>
<thead>
<tr>
<th>Mass Spectrometer</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOF-TOF</td>
<td>high resolution, high m/z fragment ions</td>
<td>Large size</td>
</tr>
<tr>
<td>keV CID</td>
<td>Not ideal for continuous ionization source</td>
<td></td>
</tr>
<tr>
<td>easier de novo peptide sequencing</td>
<td></td>
<td>$$$</td>
</tr>
<tr>
<td>dₙ and wₙ to distinguish Ile/Leu</td>
<td></td>
<td>MALDI source</td>
</tr>
</tbody>
</table>
TOF Quadrupole

Linear Ion Trap

Orbitrap

FTICR Quadrupole
Quadrupole (Q)

http://www.files.chem.vt.edu/chem-ed/ms/quadrupo.html
Quadrupole (Q)

- four parallel rods or poles
- fixed DC and alternating RF voltages

\[
\begin{align*}
\text{rf voltage} & \quad \text{+dc voltage} \\
\text{rf voltage} & \quad \text{180° out of phase} \\
\text{-dc voltage} & \quad
\end{align*}
\]

- only particular \( m/z \) will be focused on the detector, all the other ions will be deflected into the rods
- scan by varying the amplitude of the voltages
  - (AC/DC constant).
Quadrupole Field Animation

http://www.kettering.edu/~drussell/Demos/MembraneCircle/Circle.html
negative DC offset

positive DC offset

-DC

+DC
Ion Motion in Quadrupoles
- a qualitative understanding

- + DC, ions focused to center

- - DC, ions defocused

- + DC w/ rf, light ions respond to rf, eliminated (high mass filter)

- - DC w/ rf, light ions respond to rf, focused to center (low mass filter)
Quadrupole animation

http://www.youtube.com/watch?v=pjCun7QF19U
Initial kinetic energy affects ion motion in quad
Figure 9. The a-q stability diagram: a) The shaded area represents those areas in a-q space which correspond to stable solutions of Mathieu's differential equation. B) The one amu bandpass mass filter: Notice that only ions of \( m/e \ m+1 \) fall within the stability diagram.

Quadrupole Typical Specs

- **m/z Range:** 2-4000 u
- **Resolution:** Unit
- **Mass Accuracy:** ca +/− 0.1 u
- **Scan Speed:** 4000 u/s
- **Vacuum:** $10^{-4} – 10^{-5}$ Torr
- **Low Voltages:**
  - RF ~6000 –10000 V
  - DC ~500V-840V
  - Source near ground
- **Quantification:** good choice
- **Positive and Negative Ions**
- **Variations:** SingleQ, TripleQ, Hybrids
<table>
<thead>
<tr>
<th>Feature</th>
<th>TOF</th>
<th>Quad</th>
<th>3D Trap</th>
<th>Linear Trap</th>
<th>FT-ICR</th>
<th>Orbitrap</th>
</tr>
</thead>
<tbody>
<tr>
<td>mass range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resolution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>exact m/z</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all ion detection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ion storage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>speed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dynamic range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>quantification</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS/MS types</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sample introduction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>simplicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cost/performance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
What MS/MS instruments can be produced from Q?

What MS/MS instruments can be produced from Q and TOF?
Triple Quadrupole
- since 1970’s and still going strong!

Attractive Features:

- Source near ground and operates at relatively high pressure
  Couples well to source and to chromatography
- Multiple scan modes easy to implement
Triple Quadrupole (QQQ)

c/o Thermo Finnigan Corp.

c/o Agilent Corp.
Quadrupole Time of Flight (Q-TOF)

The Q-ToF™ combines a quadrupole mass filter [MS 1], a hexapole collision cell and an oTOF mass analyser [MS 2] to deliver high performance MS-MS.
Low-energy (eV) Collisions with Gas

80 fmol BSA digest

Q-TOF

QQQ
QTOF with Ion Mobility
Ion Mobility

http://bowers.chem.ucsb.edu/theory_analysis/ion-mobility/index.shtml
Disadvantages of MS

- Expensive
- Large/heavy
- No direct molecular shape information
- Requires vacuum
Ion Mobility

Ion transit through bath gas in presence of applied electric field

- **Analytical** (examples on following slides)
  - Airport security (explosives)
  - Industrial processes
  - Military uses including chemical warfare detection
  - Drugs of abuse

- **Biological** (usually coupled with m/z measurement)
  - Protein/peptide shape
Ion Mobility Process – Sample Introduction & Ionization

Ion Mobility Process – Injection into Drift Tube

Ion Mobility Process –
Separation

Ion Mobility Process – Detection

Mobility Spectrum

Principles of Mobility Separation

\[
\frac{L}{t} = v = KE
\]

\[
K = \left( \frac{3q}{16N} \right) \left( \frac{2\pi}{\mu k_B T} \right)^{1/2} \left( \frac{1}{\bar{\Omega}} \right)
\]

- \( K \) – mobility, cm²/V-s
- \( K \) reported as reduced mobility, \( K_0 \)
- \( K \) related to collisional cross section, \( \Omega \)
- **Low E/N only**

Indicative of size and shape – compare to computationally calculated cross sections

QTOF with Ion Mobility
Selection of $[\text{M}+2\text{H}]^{+2}$ at $m/z$ 246.1
Transfer CID of $[M+2H]^{+2}$ at $m/z$ 246.1
Ion mobility tutorial

https://zenodo.org/record/3268737#.XR_wCXBKi70

Differential Mobility Analyzers (DMA)
  J. Fernandez de la Mora (< 1998)
  Aerosol sciences

Travelling wave IMS (TWIMS)
  K. Giles
  Waters SYNAPT (2006)
  Structural biology

Commercial DTIMS
  PNNL designs (R.D. Smith)
  Agilent 6560 IMS-Q-TOF

Trapped ion mobility spectrometry (TIMS)
  M. Park, M. Rigdeway, F. Fernandez-Lima
  Bruker timsTOF Pro (nano-LC ion mobility Q-TOF)
What is small, inexpensive and still gives great MSMS?

Quadrupole Ion Traps

Miniature IT: Cooks, R. G. and coworkers
http://www.chem.wm.edu/dept/faculty/jcpout/faculty.html

Ion path in a trap
RF ION TRAP ELECTRODE STRUCTURES

LCQ-Type 3D Quadrupole Trap

LTQ-Type (2D) Linear Quadrupole Trap
2D vs. 3D Ion Traps
Linear Trap Demo
## Overall Performance Gains

<table>
<thead>
<tr>
<th></th>
<th>LTQ</th>
<th>3D Traps</th>
<th>Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trapping efficiency</td>
<td>~ 55-70%</td>
<td>~5%</td>
<td>~ 11-14x</td>
</tr>
<tr>
<td>Detection efficiency</td>
<td>~ 50-100%</td>
<td>~50%</td>
<td>~ 1-2x</td>
</tr>
<tr>
<td>Trapping capacity</td>
<td>~ 20,000 ions</td>
<td>~500 ions</td>
<td>~ 40x</td>
</tr>
</tbody>
</table>
9 Protein Mixture

![Graph showing the number of peptides for different gradients and time periods for Deca XP plus and LTQ.](image)
Motivating Factors Realized

- Increased Trapping Efficiency
- Increased Trapping Capacity

Which means….

- Increased Sensitivity
- Increased Inherent Dynamic Range
  - Increased S/N for full Scan MS
  - Practical MS^n
- Faster Scan Times - no µscans (only one)
How are 3-D traps and linear ion traps used in MS/MS?

Stand alone

3-D traps
LTQ

Front or back end of tandem-in-space
LT-TOF
LT-FT, LT-Orbitrap,
QTrap
Activation:

CID
Low-energy (eV) Collisions with Gas

IRMPD
(Infrared Multiphoton Dissociation)

ETD
(electron transfer dissociation)
<table>
<thead>
<tr>
<th></th>
<th>TOF</th>
<th>Quad</th>
<th>3D Trap</th>
<th>Linear Trap</th>
<th>FT-ICR</th>
<th>Orbitrap</th>
</tr>
</thead>
<tbody>
<tr>
<td>mass range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resolution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>exact m/z</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all ion detection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ion storage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>speed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dynamic range</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>quantification</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS/MS types</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sample introduction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>simplicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cost/performance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
General Conclusions

• Many instruments are complementary
• More Activation methods, the better
• Assess your needs
• Assess your budget
• @ Research level, may want to build your own