An Introduction to Mass Spectrometry

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What is a Mass Spectrometer?

Sample Introduction

Ion Source

Mass Analyser

Detector

Data System

LC, GC etc.

Mass Spectrometer
Atmospheric Pressure Ionisation (API)
- A term for any ionisation process that is carried out at atmospheric pressure

The most common modes of API are...
- Electrospray Ionisation (ESI)
  - Most commonly used
- Atmospheric Pressure Chemical Ionisation (APCI)
  - Less commonly used than ESI but still significant usage
- Atmospheric Pressure Photo Ionisation (APPI)
  - A more niche ionisation mode for certain compounds that will not ionise by either ESI or APCI.
Electrospray Ionisation (ESI)
Electrospray Ionisation Overview
Electrospray Ionisation Producing Charged Droplets

Liquid Flow

Electrospray Probe Tip at high voltage

Taylor Cone

More Negative Ions than Positive Ions

More Positive Ions than Negative Ions

Positively Charged Droplets
Droplets produced from the spray have a surface charge

Surface charged droplets undergo solvent evaporation and droplet fission to produce smaller droplets

Like charge repulsion becomes greater than droplet surface tension and fission occurs to produce smaller charged droplets
Electrospray Ionisation
Formation of Gas Phase Ions

- Ion Desorption Mechanism

- Charged Residue Mechanism
Electrospray Ionisation
Z-spray Ion Source

- Sample
- Nebulizer Gas
- Desolvation Gas
- Sample Cone
- Isolation Valve
- Extraction Cone
- Source Exhaust
- Cone Gas
- Roughing Pump
- Turbomolecular Pump
- RF Lens (Hexapole)
- Quadrupole
- Atmospheric Pressure
- Moderate Vacuum
- Full Vacuum
Atmospheric Pressure Chemical Ionisation (APCI)
APCI – Overview

- Liquid flow is forced through a narrow capillary to give it a high linear velocity
- The APCI Probe heater combined with nebulizer gas then vaporises the liquid flow
- The solvent and analyte vapour passes through the corona discharge region to produce gas phase ions
Atmospheric Pressure Photo Ionisation (APPI)
APPI – Overview

- APPI uses the same probe as APCI with a Vacuum UV light source instead of the corona discharge needle.
- As with APCI:
  - Liquid flow is forced through a capillary to give it a high linear velocity.
  - The probe heater and nebulizer gas vaporise the liquid flow.
- Ionisation occurs by either direct or chemical ionisation type processes in the region of the light source.

Diagram:
- Sample Solution
- Probe Heater
- Flash Vaporisation
- Nebulizer Gas
- Solvent and Analyte Vapour
- Vacuum UV Source
- Repeller Electrode
- Sample Cone
ESI / APCI / APPI Comparison

The diagram illustrates the comparison between ESI, APCI, and APPI techniques. It shows the range of molecular weights (y-axis) and polarity (x-axis) covered by each technique. ESI covers the highest molecular weights and all polarity ranges, including very polar compounds. APCI and APPI overlap significantly, with APCI having a slightly wider range of molecular weights and APPI covering a broader range of polarity.
Universal Source platform

Also compatible with 3rd party source options:

*Phytronix LDTD — IonSense DART*
*Prosolia DESI — Advion NanoMate — Protea LAESI*
Quadrupole Theory

- Pre-filter
- Quadrupole Mass Filter
- Post-filter

Rejected Ions  Stable (Resonant) Ions
Single Quadrupole MS
Tandem Quadrupole MS
Transfer Region Ion Optics

Maximising signal
Maximising robustness

Electric Field

Diffuse Ion Cloud
Traveling Wave Ion Transport
Traveling Wave Ion Transport

Time

Ions

Traveling Wave Pulse
Different Types of Experiments

Collision Cell

MS1

MS2
MS2 Scan

- Works in the same way as MS Scan except:
  - MS1 and Collision Cell act only as Ion guides
  - MS2 is the scanning quadrupole
- Minimises distance and amount of ion optics to interact with between mass resolved ions and the detector
- Better performance for fast scanning (> 2,000 Da/sec)
SIR – Single Ion Recording

- Only selected ions are transmitted through the instrument and are monitored constantly.
- Due to the instrument not acquiring other ions considerably more time is spent on each selected ion making this a much more sensitive acquisition mode than scanning acquisition modes.
- No mass spectra are produced for this type of acquisition.
S/N = 109
S/N = 38
S/N = 101
S/N = 129

10ng/mL of each component
A selected ion is transmitted through the first quadrupole (precursor ion), fragmented in the collision cell, and a specified fragment ion is then transmitted through the second quadrupole (product ion).

- More selective and sensitive than SIR due to
  - Specific transition needed for response to be seen
  - Less interference by background ions of the same mass

- No mass spectra are generated by MRM experiments
Selected ions are transmitted through the first quadrupole (precursor ions) and fragmented in the collision cell. MS2 is then scanned over a user defined mass range. A mass spectrum of the product ions generated by fragmentation is acquired at each time point throughout the acquisition.
Product Ion Scanning - Example
MS1 is scanned over a specified mass range and all ions are sequentially passed through to the collision cell where they are fragmented.

MS2 is set to transmit only the mass of a specific fragment ion and does not scan.

Any ions that fragment to give the specified product ion will generate a result.
Precursor Ion Scanning - Example

Precursors of 156 Da

Precursors of 85 Da

Precurso r Ion Mass Spectra

TIC

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- MS1 is scanned over a specified mass range and all ions are sequentially passed through to the collision cell where they are fragmented.
- MS2 is scanned in sync with MS1 over the same mass range minus an offset.
- A response is only seen if a precursor ion loses a neutral fragment in the collision cell of the same mass to charge ratio as the offset between MS1 and MS2.
Waters Quadrupole Mass Spectrometers

SQD 2

Xevo TQD

Xevo TQ-S micro

Xevo TQ-S
Time-of-flight mass spectrometry (ToF-MS) is probably the simplest method of mass measurement to conceptualise, although there are hidden complexities when it comes to higher resolution instruments.

The inherent characteristics of ToF MS are extreme sensitivity (all ions are detected), almost unlimited mass range, speed of analysis (modern instruments can obtain >10 full spectra per second) and sub 5ppm mass accuracy.
A voltage pulse applied to a pusher plate (typically, 800-1000V) initiates an orthogonal acceleration TOF (oa-TOF) m/z measurement.

The energy imparted by this pulse in combination with an electric field is converted into kinetic energy (KE).

The time it takes an ion to travel (TOF) a given distance (d) is proportional to its mass-to-charge ratio or m/z.
Time Of Flight Theory

Pusher

Detector

Time from Push
Accelerating the Ions

**Ideal Case:**
perfectly straight line
no orthogonal velocity component

**Spatial distribution:**
some ions are closer to the plate than others

**Energy distribution:**
small orthogonal velocity component
Waters TOF Mass Spectrometers

Synapt G2-Si HDMS

Xevo G2-XS QTof
LC/MS\textsuperscript{E} Data Acquisition

Collision cell cycles between low (MS of peptides, 4V) and elevated (MS\textsuperscript{E} of fragments, 20 \rightarrow 45 V) energy

- Global analysis: acquire data on all the ions
- Maximize the LC/MS duty cycle
- Minimize bias/selection of ions (\uparrow Reproducibility)
- Obtain qualitative and quantitative information from the same analysis run
SYNAPT G2-Si HDMS
Ion mobility
The mobility of an ionised molecule is dependant on its...

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"Classic" Ion Mobility Spectrometry

The Bowers Group Website [Ion Mobility Theory]:
http://bowers.chem.ucsb.edu/theory_analysis/ion-mobility/index.shtml
TriWave Device

- Trap
- He Cell
- IMS
- Transfer
iNFORMATION
Selectivity (and Specificity)

Retention \times CCS \times Mass = Peak capacity

Acquity UPLC
QuanTof
UPLC/HDMS
Mass Detection At Its Most Advanced
Know your peaks
Complementary & Compatible

- Information-rich mass spectral data
- Targeted for small molecule applications
- Complementary to and compatible with PDA
  - UPLC, UPC², purification
  - Optional ISM module to split and dilute
- Intuitive system for chromatographers

30-1250 DA • PRE-OPTIMIZED ES± • 20 HZ FS • 100 HZ SIR • 4 ORDERS • INTUITIVE

Waters
THE SCIENCE OF WHAT’S POSSIBLE.
6.5 mins

22 mins
Thank You

Questions?