Tendenze nell’innovazione della strumentazione in spettrometria di massa:

- Trappola lineare, orbitrap, ion mobility e nuova strumentazione
Ion Trap
Mass Spectrometry

Ion trap has been developed by the Bonn group in the early sixties. It represents one of the most simple devices (from the hardware point of view!!) with a series of capabilities which make it unique.

Analizzatori quadrupolari

Alcuni dispositivi basati su campi quadrupolari studiati dal gruppo di Paul (Univ. Di Bonn) negli anni sessanta.
Quadrupole Mass Analyzers

Differential Equations of Motion

\[
\dddot{z} - \left(4e / m(r_0^2 + 2z_0^2)\right)(U - V \cos \Omega t)z = 0
\]

\[
\dddot{r} + \left(2e / m(r_0^2 + 2z_0^2)\right)(U - V \cos \Omega t)r = 0
\]

Mathieu Equation

\[
\frac{d^2u}{d\xi^2} + [a - 2q \cos (2\xi)]u = 0, \text{ where } \xi = \Omega t/2
\]

\[
u(\xi) = A \sum_{n=-\infty}^{\infty} C_{2n} \cos(2n + \beta_u)\xi + B \sum_{n=-\infty}^{\infty} C_{2n} \sin(2n + \beta_u)
\]

\[
\omega = \frac{1}{2} \beta \cdot \Omega, \text{ where } \omega = \text{ion frequency of motion}
\]
The ion motion inside an ion trap will depend by:
- the m/z ratio of the trapped ions;
- The ion trap dimension (r);
- The dc voltage applied to the intermediated electrode U;
- The rf voltage applied to the intermediate electrode V;
- The rf frequency $\Omega$.

It can be convenient to express the stability conditions of an ion inside the trap by introducing two quantities which consider all the above parameters

$$a = \frac{-16zU}{mr^2\Omega^2}$$

$$q = \frac{8zV}{mr^2\Omega^2}$$
Apply Waveform: Excite and Fragment

Summary: Conceptual Ion Trap Operation
• **Ion trap limits:**
  • They are mainly due to space charge effects;
  • An “optimum” number of ions must be present inside the trap for its effective operative conditions;
  • The limitation of ion number inside the trap reflects on sensitivity limitation

• **Possible solution:**
  • “**Linear**” ion traps (**LIT**)
Angled view of three sections of the two-dimensional LIT. The detector faces the center section.
Ion Ejection – 2D vs 3D

Myoglobin Tryptic Digest – 1fmol  LCMS/MS
• By LIT an effective increase of sensitivity has been achieved.
• To increase selectivity a further step is required, based on the development of systems operating in high resolution.

Simulation

Peptide mixture: [Val²]-Angiotensin II Lys-des-Arg⁴-Bradykinin
Sequence: DRVYVHPF KRPPGFSPF
Formula: C₄₉H₆₉N₁₃O₁₂ C₅₀H₇₃N₁₃O₁₁
Exact mass: [M+2H]²⁺ = 516.76671 [M+2H]²⁺ = 516.78490
Δm (mmu): 18.2 mmu

RP = 18,000

Intens. (%) 100

516.65 516.70 516.75 516.80 516.85 516.90
m/z

516.77581 (observed) 516.76671 (correct) 516.78490 (correct)

RP = 56,700

Intens. (%) 100

516.65 516.70 516.75 516.80 516.85 516.90
m/z

516.76671  516.78490
Finnigan LTQ FT - FTICR MS Means:

- Fourier Transform
- Ion Cyclotron Resonance
- Mass Spectrometry
- The mathematical method applied to convert the acquired transient signal into a frequency spectrum
- The way masses are separated different masses have different cyclotron resonance frequencies in a magnetic field
- The analytical technique

opening up unlimited possibility
on lc/ms/ms
La trasformata di Fourier

\[ X(f) = \int_{-\infty}^{+\infty} x(t) \cdot e^{-j2\pi ft} \, dt \]

\[ x(t) = \int_{-\infty}^{+\infty} X(f) \cdot e^{j2\pi ft} \, df \]
La serie di Fourier

Un polinomio trigonometrico è una funzione periodica di periodo $2\pi$ definita sul campo reale del tipo:

$$f(t) = \frac{a_0}{2} + \sum_{n} [a_n \cos(nt) + b_n \sin(nt)] = \sum_{n} c_n e^{int}$$

doce $a_n$ e $b_n$ sono numeri reali, $c_n$ complessi e $n$ è intero.

In matematica, in particolare in analisi armonica, la serie di Fourier è una rappresentazione di una funzione periodica mediante una combinazione lineare di funzioni sinusoidal fundamentali:

$$x_n \mapsto c_n e^{int}$$

Tali funzioni sono i vettori di base del sistema ortonormale trigonometrico, e sono le potenze di $e^{it}$. Attraverso la formula di Eulero possono essere espresse in modo equivalente con le funzioni seno e coseno. Un tale tipo di decomposizione è alla base dell’analisi di Fourier.

[Diagramma della serie di Fourier con l'approximazione di una funzione onda quadra attraverso i primi quattro termini della corrispondente serie di Fourier.]
Attention must be paid with the Fourier transform!
Linear Ion Trap – FTICR Hybrid

The Orbitrap: a new mass spectrometer

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Electrostatic Traps

“...Static charges can not be stable in electrostatic fields...”

Linear traps  Segmented-ring traps
Orbital traps
Trajectories in the orbitrap

- Characteristic frequencies:
  - Frequency of rotation \( \omega_\phi \)
  - Frequency of radial oscillations \( \omega_r \)
  - Frequency of axial oscillations \( \omega_z \)

\[
\begin{align*}
\omega_\phi &= \frac{\omega_z}{\sqrt{2}} \sqrt{\left(\frac{R_m}{R}\right)^2 - 1} \\
\omega_r &= \omega_z \sqrt{\left(\frac{R_m}{R}\right)^2 - 2} \\
\omega_z &= \sqrt{\frac{k}{m \omega}}
\end{align*}
\]

Lord of the ion rings: Forging of the ring

Electrodynamical Squeezing

Lord of the ion rings: Retainment of the rings

\[ \omega = \sqrt{\frac{k}{m/z}} \]

1. Frequencies are determined using a Fourier Transformation
2. For higher sensitivity AND resolution, transients should not decay too fast

Ultra-high vacuum Ultra-high precision

Figure 3. (a) Typical transient acquired to record the mass spectrum of bovine insulin. The transient acquired is equivalent to the free induction decay of FT NMR experiments. Top shows an expanded portion of the transient.
LTQ-Orbitrap: 2nd generation of the “fast” injection (orthogonal)

1. Ions are stored in the linear trap
2. …are axially ejected
3. …and trapped in the C-trap and squeezed into a smaller cloud
4. …then a voltage pulse across C-trap ejects ions towards the Orbitrap
5. …where they are trapped and detected

Orthobi (2004)-Thermo Bremen; Prototypes/Series

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**Figure 5.** ESI mass spectrum of bovine insulin. Data acquisition parameters include a data sampling rate of 5 MHz; record length was 8 million data points, and the Fourier transform was performed with no apodization function or zero-filling. The lower spectrum shows a wide range mass spectrum including the internal mass calibrant Ultraspec 1621 whose oligomers are spaced by 100 mass/charge unit intervals. Lower traces in the close-ups show experimentally obtained isotopic distributions for each charge state. Upper traces in the close-ups show the theoretically expected isotopic distributions. The calculated isotope distributions were obtained from IsoPro 3.0 using Gaussian peak shapes with resolution of 100,000.
Introduction to ion mobility methods

Figure 13. ESI mass spectrum of hexa-N-acetyl chitohexaose. Inset shows a close-up of the experimental (lower trace) and theoretically expected (upper trace) isotopic distributions. Filled squares denote ions with structure [(saccharide)_nOH]^+, while open squares denote fragment ions with structures [(saccharide)_mH]^+.
To perform an ion mobility experiment, ions are introduced into an atmospheric pressureregion(called “drift tube”) across which an electric field is uniformly applied. The uniform field is generated by connecting a series of evenly spaced rings with equal value resistors.

The time required for the ions to reach the detector depends upon the ion’s
- Collision cross section (averaged over all possible orientation of the ion);
- charge state;
- mass;
- drift tube operating parameters (electric field strength, drift tube length, buffer gas pressure, temperature)
Some definitions:

**Reduced ion mobility**

\[
K_0 = \frac{L \cdot P \cdot 273}{t_D \cdot E \cdot 760 \cdot T}
\]

Where \(t_D\) is the drift time, \(L\) the length of the drift tube, \(P\) the pressure, \(E\) the electric field strength and \(T\) the temperature.

**Collision cross section**

\[
\Omega = \frac{(18\pi)^{1/2}}{16} \frac{ze}{(k_BT)^{1/2}} \left( \frac{1}{m_I} + \frac{1}{m_B} \right)^{1/2} \frac{t_D E \cdot 760}{L \cdot P \cdot 273} \frac{T}{N}
\]
Protein conformation

Measuring the ion mobility of an ion can yield information about its structure as small, compact, ions drift quicker than large extended ions.
Flexibility

- Fragmentation can be induced in both TRAP and TRANSFER T-WAVES
- The system can operate in both Mobility-Tof and Tof only mode
ESI Mass Spectrum of $\alpha$-Lactalbumin
Excitation in Trap T-Wave results in a more open conformation.

High efficiency ion mobility
Something new!
Direct analyses on living bodies*

*The lecturer advise against to try to perform these experiments by yourselves
Figure 1. Experimental setup of MV electrostatic ionization mass spectrometry. A living individual was electrically treated to MV potential by putting one or both hands on the metal ball of the Van de Graaff generator for the gradual accumulation of charge for about 2 s. Then the other hand of the individual was moved in front of the extended inlet of the mass spectrometer. Chemicals on the surface of the living individual were ionized by the MV potential, and desorbed toward the MS inlet for detection. The Van de Graaff generator (with positive potential at 60 MV or negative potential at 5.4 MV) was switched on by pressing a button. After the individualisation was over, the instrument was switched on and the mass spectrum was monitored. To prevent electrical damage, the instrument was shielded from electrostatic charge using several electrically grounded metal sheets. After the individual was ionized, an extended inlet was used to extend the ion source inlet away from the ion source block for easier sampling. The ion source was electrically grounded to prevent the accumulation of electrostatic charge on the ion source.

Figure 2. Schematic shows the use of MV electrostatic charging for ionization and mass spectrometric analysis of a human body. The living individual was charged electrostatically to MV potential (0.4 MV) at negative polarity by touching a Van de Graaff generator. An explosive compound (1 mg of 2,4-dinitrotoluene, 2,4-DNT) deposited on the latex glove was readily ionized and desorbed, and then detected by a mass spectrometer. (a) The 2,4-DNT in the form of deproteinized ion at m/z 181.0; (b) its corresponding tandem mass spectrum showing the detection of characteristic fragment ions at m/z 151.0 and 135.0, corresponding to the neutral loss of NO and NO2, respectively.
Figure 4. Direct detection of exogenous compounds in the breath of a healthy individual, after chewing mint-flavored gum and drinking a cup of red wine, respectively. Mass spectra of the breath background before (a) chewing of mint-flavored chewing gum and (b) drinking red wine, de Mass spectrum of breath after chewing mint-flavored gum for 5 min. Ions at m/z 313.3 in (b) were assigned to be the protonated menthol dimer (Ment + H = Ment^+), ions at m/z 293.2 were assigned to be the protonated adduct of menthol with a background molecule (i.e., (Ment + H + Bg^+)), their corresponding tandem mass spectra are shown in Supplementary Figure S3. (c) Mass spectrum of breath after drinking red wine. Ions at m/z 178.1 were assigned to be protonated ethanol butyrates, which is a volatile component in red wine [14]. The corresponding tandem mass spectrum of protonated ethanol butyrates is shown in Supplementary Figure S4.
Dedication

To ion trappers, young and old, everywhere.