Modern Mass Spectrometry

MacMillan Group Meeting
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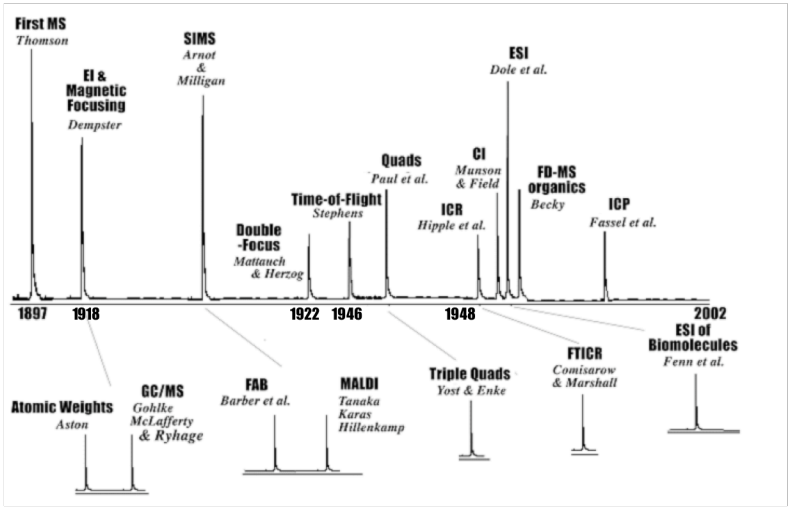
Sandra Lee

Key References:

E. Uggerud, S. Petrie, D. K. Bohme, F. Turecek, D. Schröder, H. Schwarz, D. Plattner, T. Wyttenbach, M. T. Bowers, P. B. Armentrout, S. A. Truger, T. Junker, G. Suizdak, Mark Brönstrup. *Topics in Current Chemistry: Modern Mass Spectroscopy*, pp. 1-302, 225. Springer-Verlag, Berlin, 2003.

Current Topics in Organic Chemistry **2003**, *15*, 1503-1624

Mass Spectroscopy: A Historical Perspective



The Basics of Mass Spectroscopy

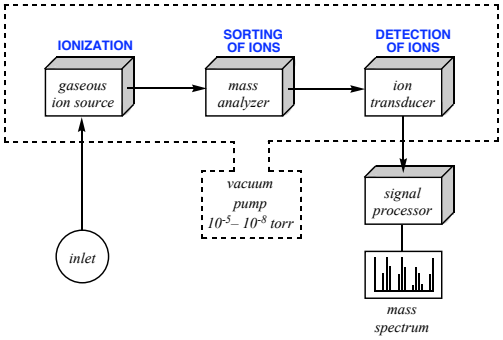
Purpose

Mass spectrometers use the difference in mass-to-charge ratio (*m*/*z*) of ionized atoms or molecules to separate them. Therefore, mass spectroscopy allows quantitation of atoms or molecules and provides structural information by the identification of distinctive fragmentation patterns.

The general operation of a mass spectrometer is:

1. create gas-phase ions
2. separate the ions in space or time based on their mass-to-charge ratio
3. measure the quantity of ions of each mass-to-charge ratio

Instrumentation



Ionization sources

Chemical Ionisation (CI)
Atmospheric Pressure CI (APCI)
Electron Impact (EI)
Electrospray Ionization (ESI)
Fast Atom Bombardment (FAB)
Field Desorption/Field Ionisation (FD/FI)
Matrix Assisted Laser Desorption Ionisation (MALDI)
Thermospray Ionisation (TI)

Analyzers

quadrupoles
Time-of-Flight (TOF)
magnetic sectors
Fourier transform
and quadrupole ion traps

Detectors

electron multiplier
Faraday cup

Ionization Sources: Classical Methods

Electron Impact Ionization

A beam of electrons passes through a gas-phase sample and collides with neutral analyte molcules (M) to produce a positively charged ion or a fragment ion. Generally electrons with energies of 70 ev are used.

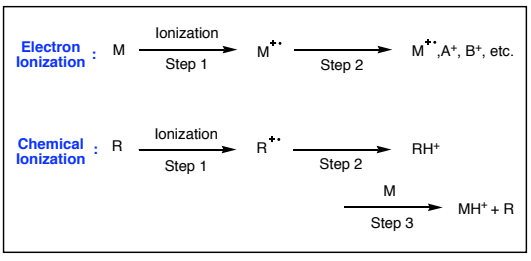
This method is applicable to all volatile compounds ($>10^3$ Da) and gives reproducible mass spectra with fragmentation to provide structural information.

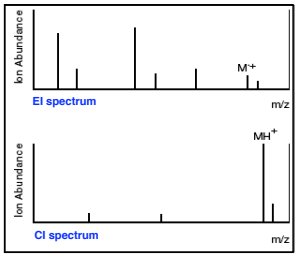
Chemical Impact Ionization

Ionization bergins when a reagent gas (R) is ionized by electron impact and then subsequently reacts with analyte molecules (M) to produce analyte ions.

This method gives molecular weight information and reduced fragmentation in comparison to EI.

reagent gas (R)	molecular ion	reactive reagent ion
H_2	$H_2^{+\bullet}$	H_3^+
C_4H_{10}	$C_4H_{10}^{+\bullet}$	$C_4H_{11}^+$
NH_3	$NH_3^{+\bullet}$	NH_4^+
CH_3OH	$CH_3OH^{+\bullet}$	$CH_3OH_2^+$
NO	$NO^{+\bullet}$	NO^+





Ionization by Particle Bombardment

The Theory

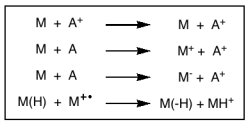
The analyte is in a liquid matrix (with low volatility) and is bombarded with high current of bombarding particles. The *primary* partile beam is the bombarding particle beam, while the *secondary ions* are the ions produced from bombarment of the liquid target surface.

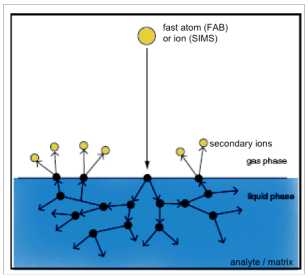
This is a soft ionization technique and suited for the analysis of low volatility species, typically producing large peaks for the pseudo-molecular ion species [M+H]+ and [M-H]-, along with structurally significant fragment ions and some higher mass cluster ions and dimers

Fast Atom Bombardment (FAB) : neutral inert gas (typically Ar or Xe at energies of 4-10 KeV) are accelerated by electric potential to give high-velocity beam of ions. ionization of a neutral molecule occurs by close encounter with a fast moving atom or ion.

Secondary Ion Mass Spectroscopy (SIMS) : ions (typically Cs^+ at energies of 2 - 30KeV) are focused and accelerated to higher KE's than is possible in FAB, so sensitivity is improved for higher masses.

solvent	protonated molecular ions (*m/z*)
gycerol	93
thiogycerol	109
3-nitrobenzyl alcohol	154
n-octyl-3-nitrophenyl ether	252
triethanolamine	150
diethanolamine	106
polyehtylene glycol (mixtures)	---



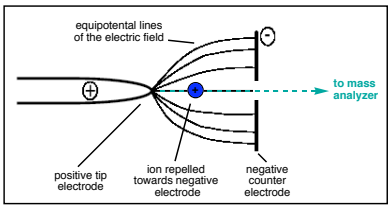


Field Ionization (FI) versus Field Desorption (FD)

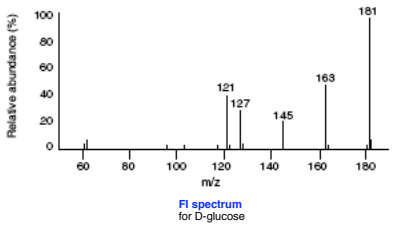
The Theory

A high electric voltage (potential) that gives rise to lines of equipotential result in an electric field crowd around the needle tip. The electric field is most intense at the surface (point) of the tip and is where ionization occurs.

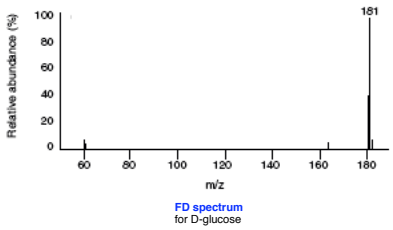
FI : sample is heated in a vacuum so as to volatilize it onto an ionization surface. FI is suited for use with volatile, thermally stable compounds. FI sources are arranged to function also as FD sources



FD : the sample is placed directly onto the surface (dipping emitter in an analyte solution) before ionization but FD is needed for non-volatile and/or thermally labile substances.



FI spectrum
for D-glucose



FD spectrum
for D-glucose

Soft Ionization Methods

- **Electrospray Ionization (ESI)**

A solution is nebulized under atmospheric pressure and exposed to a high electrical field which creates a charge on the surface of the droplet. Droplets rapidly become much smaller through vaporization of solvent and into an analyzer.

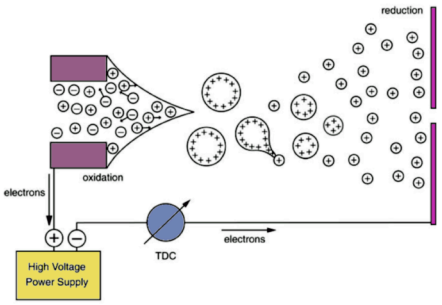
By producing multiply charged ions,electrospray is extremely useful for accurate mass measurement,particularly for thermally labile, high molecular mass substances (ie. proteins, oligonucleotides, synthetic polymers, etc.)

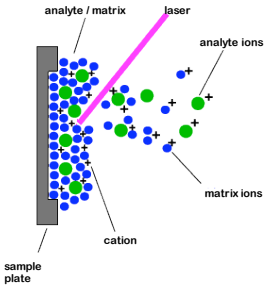
- **Matrix-Assisted Laser Desorption/Ionization (MALDI)**

Laser evaporation from a crystallized sample/matrix mixture. The matrix material must have an which has an absorption spectrum that matches the laser wavelength of energy.

Matrix acts as a receptical for the laser energy and facilitaes ionization while minimizing ablation of the sample and analyte ion that would otherwise occur from direct laser desorption.

More tolerant of salts and complex mixture analysis than ESI.

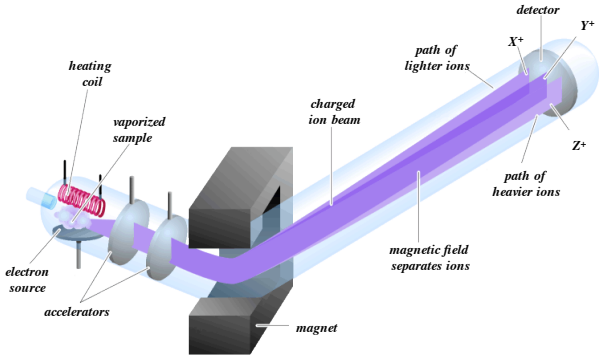




Comparing Ionization Methods

ionization method	type of ion formed	analytes	sample intro	mass limits	method type
EI	M^+, M^-	small volatiles	GC, liquid or solid probe	10^3	hard method structural info
CI	$[M+H]^+$, $[M+X]^+$	small volatiles	GC, liquid or solid probe	10^3	soft method
APCI	$[M+H]^+$, $[M+X]^+$, $[M-H]^-$	small volatiles (less polar species)	LC or syringe	$2x10^3$	soft method
FI/FD	$[M+H]^+$, $[M+X]^+$ solid probe	FI: volatiles FD: nonvolatiles	GC, liquid or solid probe	$2x10^3$	soft method
ES	$[M+nH]^{n+}$, $[M-nX]^{n-}$	peptides, proteins nonvolatile	LC or syringe	$2x10^5$	soft method multiply charged ions
FAB	$[M+H]^+$, $[M-H]^-$	carbohydrates organometallics peptides, nonvolatile	in viscous matrix	$6x10^3$	soft but harder than ESI or MALDI
MALDI	$[M+H]^+$, $[M+X]^+$	peptides, proteins nucleotides	in solid matrix	$5x10^5$	soft

The Classic: Magnetic Sector Mass Analyzer



Features

High resolution
High sensitivity
High dynamic range
High-energy CID MS/MS spectra are very reproducible
Not well-suited for pulsed ionization methods (e.g. MALDI)
Usually larger and higher cost than other mass analyzers

Ion Trajectory

Ions from the ion source are accelerated to high velocity though a magnetic sector, in which a magnetic field is applied perpendicular to the direction of ion motion.

Ion velocity then becomes constant but in a circular path at angles of180, 90, or 60°.

Ions are sorted mass to charge ratio by holding V and r constant while varying B

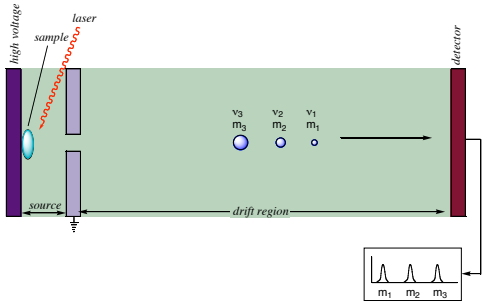
$$\frac{m}{z} = \frac{B^2 r^2 e}{2V}$$

Time-of-Flight Mass Analyzer

Features

Advantages: simplicity, ruggedness, ease of accessibilty to ion source, unlimited mass range and rapid data aquisition.

Disadvantages: Data aquisition must be fast, variations of in ion velocities can create peak broadening which can limit resolution



Ion Trajectory

Ions are generated from electron, secondary ions, or laser generated photons

Ions are accelerated by an electronic field with equal E into a field-free drift tube (L = 1m). Ions have different velocities due to differences in mass. Typical flight times are 1-30 μs.

$$v = \frac{L}{t} \qquad \frac{m}{z} = \frac{2Vt^2}{L^2} \qquad t = L\sqrt{\frac{m}{z2V}}$$

Quadrapole Mass Analyzers

Features

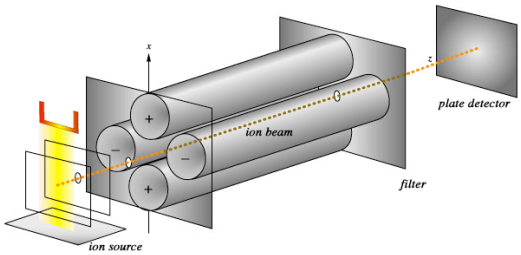
Most common system due to low scan times (100 ms), compact design, less expensive than other analyzers

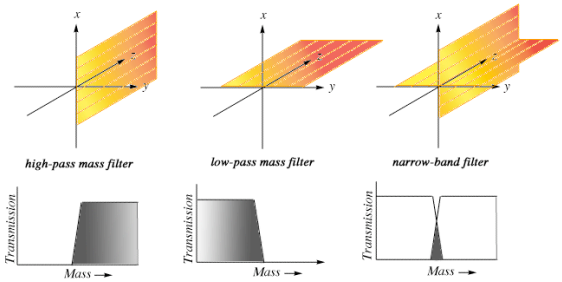
Ion Trajectory

Four parallel rods are the electrodes where are the positive and negative terminals of a dc source. Variable radio-frequency ac potentials (180 °) out-of-phase are applied to each pair of electrodes.

Ions are accelerated between the rods and must keep a stable trajectory in the *xz* plane (high pass-mass filter) and the *yz* plane (low-pass mass filter to get to the detector.

Ions that differ in one mass unit can be resolved by adjusting the center of the band by modulating the ac/dc potentials.





Fourier Transform Ion Cyclotron Resonance (FT-ICR) Mass Analyzer

Utility

Most complex method of mass analysis but most sensitive of the techniques in common use today. Almost unlimited mass resolution, >10^6 is routinely observable with resolutions in the 10^4 to 10^5 range.

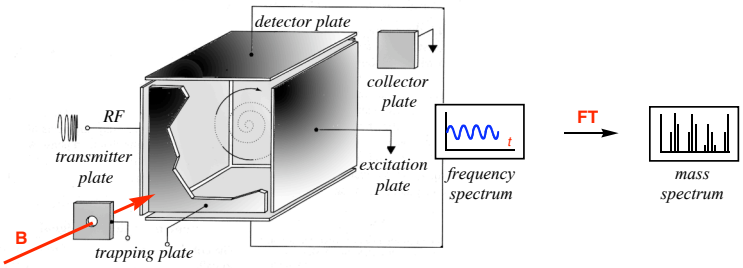
Ion Trajectory

Ions drift into a spatially uniform static magnetic field of strength, B, that causes the motion to become circular in a plane perpendicular to the direction of the magnetic field. Within this ion-trap, the angular frequency (ω_c) is inversely proportional to the *m*/*z* value.

$$F = zv \times B \qquad \omega_c = \frac{v}{r} = \frac{zB}{2\pi m} \quad \text{or} \quad \frac{m}{z} = \frac{B}{2p\omega}$$

The presence of ions between a pair of detector electrodes (in the trapping cell) will not actually produce any measurable signal. It is necessary to excite the ions of a given *m*/*z* as a coherent package to a larger orbital radius, by applying an RF sweep of a few milliseconds across the cell. One frequency will excite one particular (Fourier transformation allows for all frequencies to measure simultaneously).

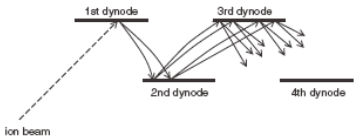
Measurement of the angular frequency leads to values for *m*/*z* and thus to the mass spectrum. Because frequency can be measured more accurately than any other physical property, the technique has a very high mass resolution.

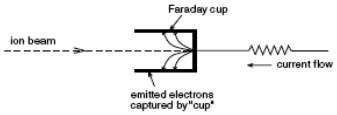


Transducers for Mass Spectroscopy: Detectors

Electron Multipliers

An incident ion beam causes two e^- to be emitted from the first dynode. These electrons are accelerated to the second dynode where each causes two more electrons (four in all) to be ejected. These in turn are accelerated to a third dynode and so on, eventually reaching, say, a tenth dynode by which time the initial two electrons have become a shower of 29 e^-'s.



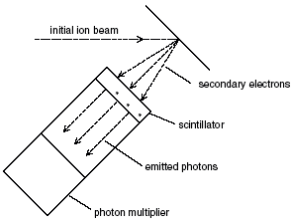


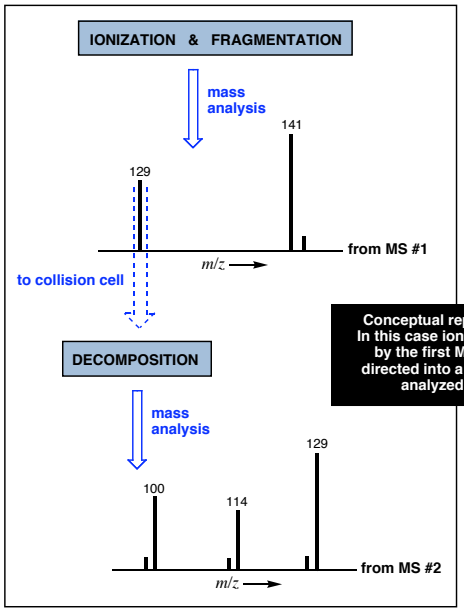
Faraday Cup

Ions travelling at high speed strike the inside of the metal (Faraday) cup and cause secondary e^- to be ejected. This production of electrons constitutes a temporary flow of electric current until the electrons have been recaptured. The Faraday cup detector is simple and robust and is used in situations in which high sensitivity is not required.

Scintillator ('Daly' detector)

A fast ion causes electrons to be emitted and these are accelerated towards a second 'dynode'. In this case, the dynode consists of a substance (a scintillator) which emits photons (light). The emitted light is detected by a photomultiplier and is converted into an electric current. Since photon multipliers are very sensitive, high gain amplification of the arrival of a single ion is achieved. These detectors are also important in studies on metastable ions.





Conceptual representation of MS/MS
In this case ions of m/z 129 is selected by the first MS and these ions are directed into a collision chamber and analyzed by a second MS.

MS/MS : Hybrid Mass Spectroscopy

Advantages of using Tandem Mass Spectroscopy:

- Allows for more powerful
- Structure elucidation
- Selective detection of a target compound
- Greatly reduce interferences
- Study of ion-molecule reactions

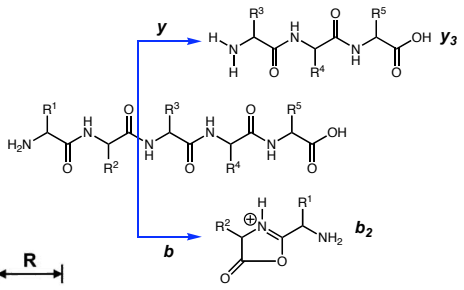
Tandem Mass Spectroscopy for Structural Biology

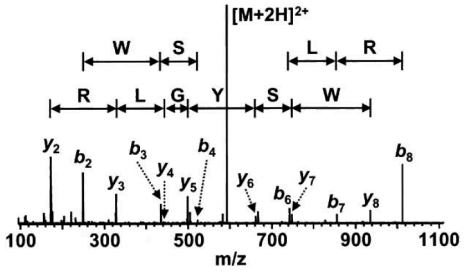
An Alternative to Traditional Sequencing Techniques

■ MS Techniques for the Specific Fragmentation of Peptides

Sustained off-resonance irradiation collision-induced dissociation (SORI-CID)
Infrared multiphoton dissociation (IRMPD)
Blackbody infrared radiative dissociation (BIRD)
Surface-induced dissociation
Electron capture dissociation (ECD)

CID is used to induce fragmentation of gas-phase ions via inelastic collisions of translationally excited ions with neutral atoms or molecules. Fragmentation of amide bonds to produce *N*-terminal ***b*** and *C*-terminal ***y*** ions is observed also in IRMPD and BIRD



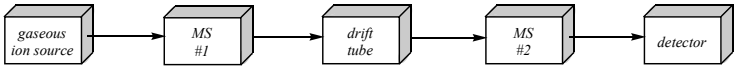


SORI-CID fragment ion spectrum of human luteinizing hormone-releasing hormone using 7 Tesla FT-ICR MS.
All amide bonds, except the two closest to the *N*- and *C*-termini, were cleaved in this 10-residue peptide, resulting in a sequence tag of six amino acid residues from the ***y*** ion series

The Ion Mobility / Ion Chromatography Method

Structural Information of the Gas Phase Conformation of Molecules or Non-Covalent Clusters

■ Ion Mobility-Mass Spectroscopy

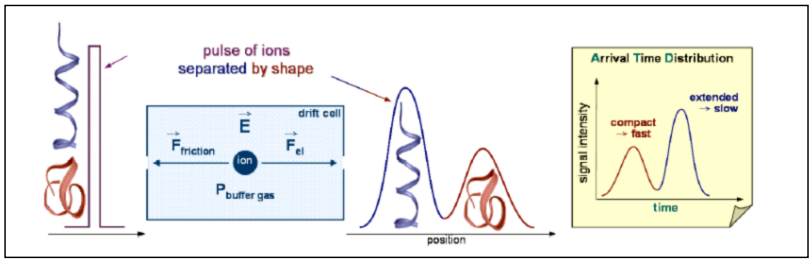


Ion mobility (K) is defined by an ions ability to travel through a buffer gas under weak uniform electric field to an equilibrium drift velocity.

$$K = \frac{3z}{16N}\left(\frac{2\pi}{\mu k_B T}\right)^{1/2}\frac{1}{\sigma}$$

Collision cross section (σ) is determined by ion geometry but also interaction between ion and the buffer gas. (ie. ions with compact structures have small cross sections and large ion mobility)

Ions are separated by size in a sort of "ion chromatography."



The Ion Mobility / Ion Chromatography Method

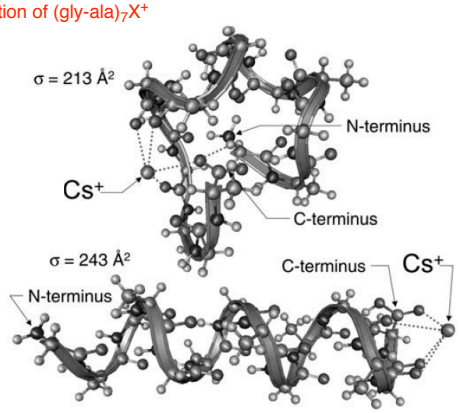
Structural Information of the Gas Phase Conformation of Molecules or Non-Covalent Clusters

■ Determining the Peptide Configuration of $(gly\text{-}ala)_7X^+$

Predicted from Ramachandrin plot :

X^+	% α-helicity
H^+	23%
Na^+	59%
K^+	77%
Rb^+	90%

Calculations predict that for $X^+ = Cs^+$ that the α–helical structure is 10 kcal•mol^{-1} more stable than the globular configuration



Ion mobility experiments show that for $X^+ = H^+$, Li^+, Cs^+ the a cross sections are all within 211-216 Å^2 at 300K which charaterizes these as globular compact zwitterionic structures.

Witt & Bowers: *JACS* **2000**,*122*, 3458.

Thermochemistry by Threshold Collision-Induced Dissociations

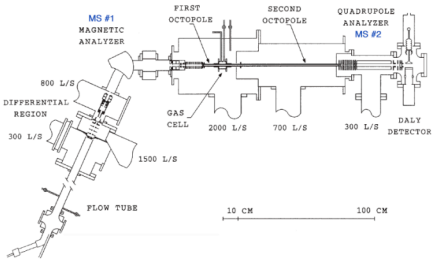
Determination of Accurate Gas-Phase Binding Energies and Reaction Barriers

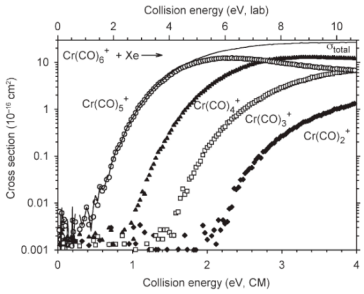
■ Threshold Collision-Induced Dissociation

Threshold collision-induced dissociation (CID), which is an intrinsically endothermic reaction, is used to determine the energy threshold of the dissociation of the molecular ion AB^+.

$$AB^+ + Xe \longrightarrow A^+ + B + Xe \quad (1)$$

The threshold measured for the reaction in eq. (1), E_0, corresponds to the highest energy along the reaction coordinate for dissociation, i.e., the activation barrier, which occurs at the transition state for the reaction.





■ Bond Dissociation Energies of $Cr(CO)_6$

The measured threshold corresponds to the bond dissociation energy (BDE) of CO from $Cr(CO)_n$.

$$D_0(A^+ - B) = E_0 \quad (2)$$

Total cross section clearly varies smoothly with energy and that CO losses are clearly sequential.

Ervin & Armentrout: *J. Phys. Chem.* **2001**,*115*, 1213.

Applications of MS to Organometallic Chemistry

Parallelling Solution Phase and Ion-molecule Reactions in Gas-phase

Theory

Short-lived intermediates of catalytic cycles can be trapped and identified and susequent ion-molecule reactions yields detailed information about single reaction steps of catalytic cycles.

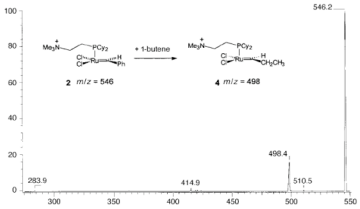
Gas-phase reactions performed to corroborate species detected by MS to solution-phase species.

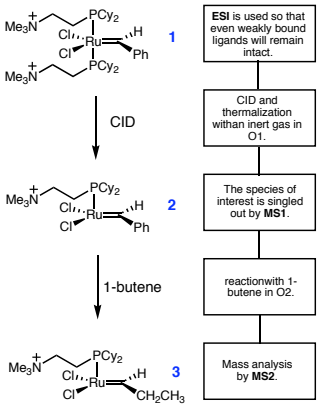
Olefin Metathesis by Ruthenium Carbene Complexes

Increasing the collisional activation potential resulted in **1** predominantly going to **2** due to loss of the second phosphine ligand, loss of trimethylamine, and loss of HCl.

The observed fragmentation pattern was consistent with the assumed structure of the ruthenium complex.

Reaction of **2** with 1-butene as reaction partner gave a new signal with the mass corresponding to the **3** via an olefin metathesis reaction.





Parallelling of gas and solution phase reactivity:

Dissociation of one phosphine ligand is a prerequiste for reactivity.

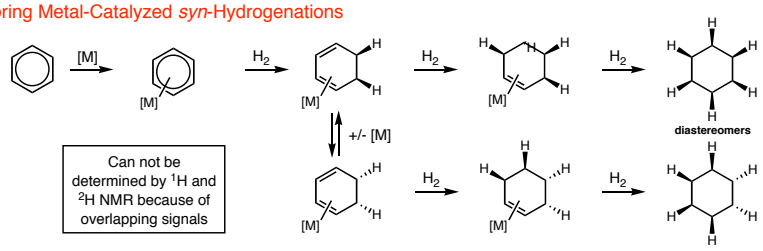
Kinetic preference for one propylidine complex (no other methylene complext detected).

Mohr,Lynn, & Grubbs: *Organometallics* **1996**, *15*, 4317.
Hinderling, Adlhart, & Chen: *ACIE* **1998**, *37*, 2685.

Diastereoselective Effects

MS Distinction of Stereoisomers in Arene Hydrogentation

Exploring Metal-Catalyzed *syn*-Hydrogenations

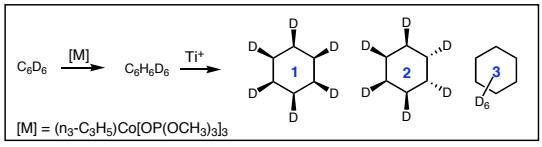


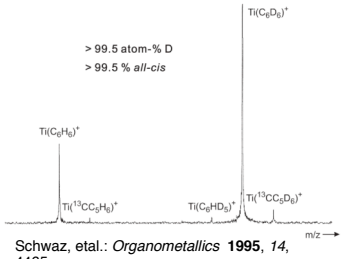
Can not be determined by ^1H and ^2H NMR because of overlapping signals

MS Stereoselective Effects by Isotopic Labeling

Diastereoselectivity of a hydrogentation is probed by using isotopic labelling and a gas-phase dehydrogenation reaction with bare Ti^+.

Partial FTICR MS of triple hydrogenation of C_6H_6 shows that >99.5%all *syn*-hydrogenation of C_6D_6 and the triple dehydrogenation of occurs with >99.5 % diastereoselectivity



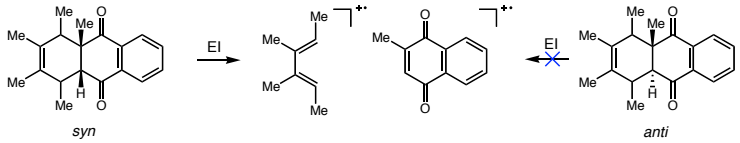


Schwaz, etal.: *Organometallics* **1995**, *14*, 4465.

Diastereoselective Effects

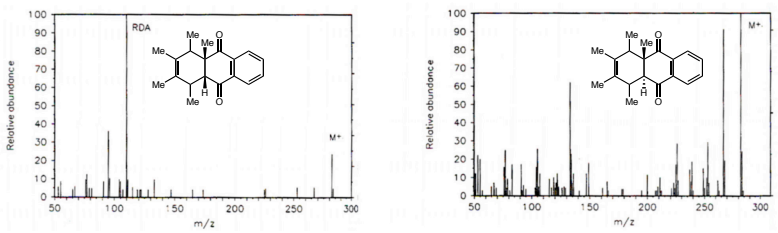
MS Distinction of Stereoisomers in Fragmentation

- Retro-Diels-Alder of Stereoisomers



EI gives highly stereospecific RDA fragmentation for isomers with cis-ring junction of cyclohexadiene

Suggestive that D-A mechanism is a concerted reaction that exhibits symmetry-conservation because a stepwise mechanism could not show such a difference between stereoisomers



Methods for the MS Quantitation of Chiral Molecules

- Chiral Recognition Based on Ion/molecule Reactions

Diastereomeric adducts, generated using chiral reference compound, is investigated in a single-stage MS experiment.

One enantiomer of the analyte is isotopically labeled so that the corresponding mixture of diastereomeric adducts can be mass resolved.

- Chiral Recognition Based on Exchange Reactions

Diastereomeric aducts, generated from a chiral ligand and a chiral host such as α-cyclodextrin (CD), is mass-selected and allowed to exchange the chiral ligand in a reaction with a neutral gas (chiral or achiral) in an MS/MS experiment.

Chiral distinction is due to the exchange rate varying with the chirality of the analyte incorporated into the adduct ion.

- Chiral Recognition Based on CID of a Diastereomeric Adduct

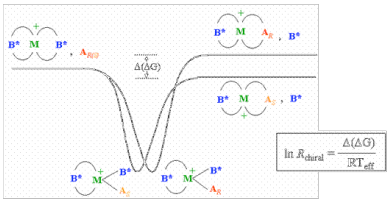
CID of diastereomeric adducts formed from an analyte and a chiral reference in a tandem massspectrometry (MS/MS) experiment. Successful experiments are limited to particular molecules, most of which are stereorigid.

- Chiral Recognition Based on "The Kinetic Method"

The kinetic method (MS/MS) to quantify the chiral effects. Involves the CID of a trimeric transition metal ion-bound cluster ion which give rise to diastereomeric product ions.

MS Quantitation of Chiral Molecules by the Kinetic Method

■ Theory of the Kinetic Method



Potential diagram for the chiral recognition of analyte A (A_R and A_S) based on the formation of diasteromeric ions through CID of a metal ion (M)-bound cluster.

B* is the chiral reference compound.

Chiral selectivity, R_{chiral} (= R_R/R_S), is related to $\Delta(\Delta G)$.

A CID of a trimeric transition metal ion-bound cluster ions (composed of three chiral ligands: one of the analyte and two of the reference compound) which give rise to diastereomeric product ions.

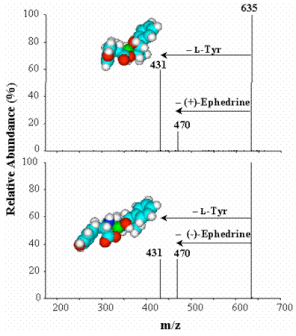
Chiral selectivity of compounds is determined by the relative abundances of the product ions caused by differences in the energy required for their formation.

A two-point calibration curve, derived from the kinetic method,allows rapid quantitation of enantiomeric excess of chiral mixtures.

$$\ln R = \frac{\Delta(\Delta G)_R + \Delta(\Delta G)_S}{2RT_{eff}} + \frac{\Delta(\Delta G)_R - \Delta(\Delta G)_S}{2RT_{eff}}\text{ee}$$

Cooks: *Anal. Chem.* **2004**, *76*, 663.
JACS **2000**,*122*, 10598.

MS Quantitation of Chiral Molecules by the Kinetic Method

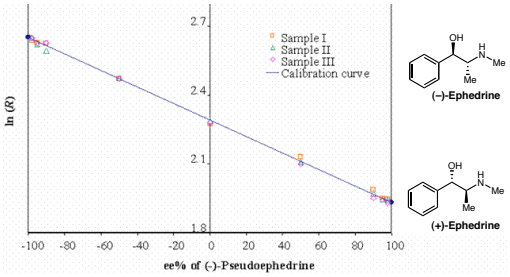


■ Resolution of Pseudoephedrine

Chiral recognition of ephedrine by the kinetic formation of diastereomeric ions [Cu(L-Tyr)(A) - H]$^+$ (m/z 431, where A is (+)-and (–)-ephedrine) via CID of [Cu(L-Tyr)$_2$(A) - H]$^+$ (m/z 635).

The common product is [Cu(L-Tyr)$_2$ - H]$^+$ (m/z 470) is the internal standard.

3-D models show a stronger π-cation interaction in one of the diastereomers.



Enantiomeric determination of pseudophedrine. The average error is 2.6 % ee for the range of –99% to 99% ee.

After calibration the measurement of one sample is less than 30 seconds.

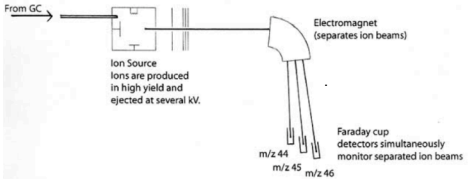
Cooks: *Anal. Chem.* **2004**, *76*, 663 & *JACS* **2000**,*122*, 10598.

Sourcing Organic Compounds Based on Isotopic Variation

High Precision Isotope Ratio Mass Spectroscopy (IRMS)

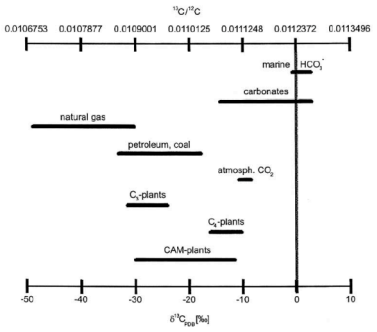
Instrumentation

IRMS instruments are highly specialized for the analysis of $^{13}C/^{12}C$, $^{2}H/^{1}H$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, and $^{34}S/^{32}S$ via analysis gases CO_2, H_2, N_2, and SO_2 using a tight electron impact ion source, high transmission magnetic sector, and multiple collectors, delivering relative standard deviations of less than 0.01%



Isotopic Fractionation as a Fingerprinting Technique

Studies over 50 years have shown that isotopic fractionation due to physiological processes, specifically CO_2 transport processes within plants and photosynthesis, leads to variation in isotope ratio in natural compounds



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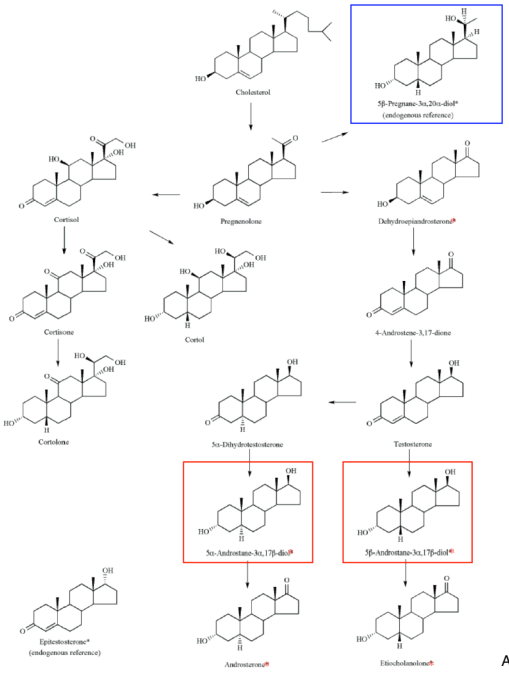

Synthetic versus Natural Steriods

Synthetically-derived steroids show lower $\delta^{13}C$ than their analogues in the human body since they are synthesized using starting materials that are extracted from plants with low ^{13}C content, mostly soy.

Endogenous steroids originates from cholesterol that is part of the human diet and are less depleted in ^{13}C.

The differences in the ^{13}C ratios between endogenous and exogenous steroids are used today to directly detect the abuse of anabolic steroids with GCC-IRMS

Detection of Synthetic Steriods

$\delta^{13}C$ ratio before and after administration of exogenous testosterone metabolites:

5β–adiol: -24.78 to -27.04% ⟶ -28.40 to 33.35%

5α–adiol: -23.68 to -27.31% ⟶ -28.53 to 31.24%

The differences between the $\delta^{13}C$ of 5β-adiol and 5α-adiol for the individual subjects were not bigger than 1.8 -1.9% before the testosterone administration and not smaller than 3.9 - 3.6% after testosterone was given. The maximum differences after administration of testosterone were 8.2 and 7.7% respectively

Aguilera, etal: *J. Chromatogr. B Biomed. Sci. Appl.*, **1999**,*727*, 95.

Modern Mass Spectroscopy: Conclusions

- MS is useful for chemists as well as researchers from neighboring disciplines such as physics, medicine, or biology as a powerful analytical tool.
- The environment-free conditions of the highly diluted gas phase allows the more direct study of a simplified system.
- The combination of isotopic labeling and MS allows for a detailed analysis of reaction mechanisms or conformational analysis through H/D exchange experiments.
- MS allows for the study of diastereomeric processes and for enantioselective quantification
- Tandem MS/MS is a poweful technology has opened an avenue for newer and broader applications