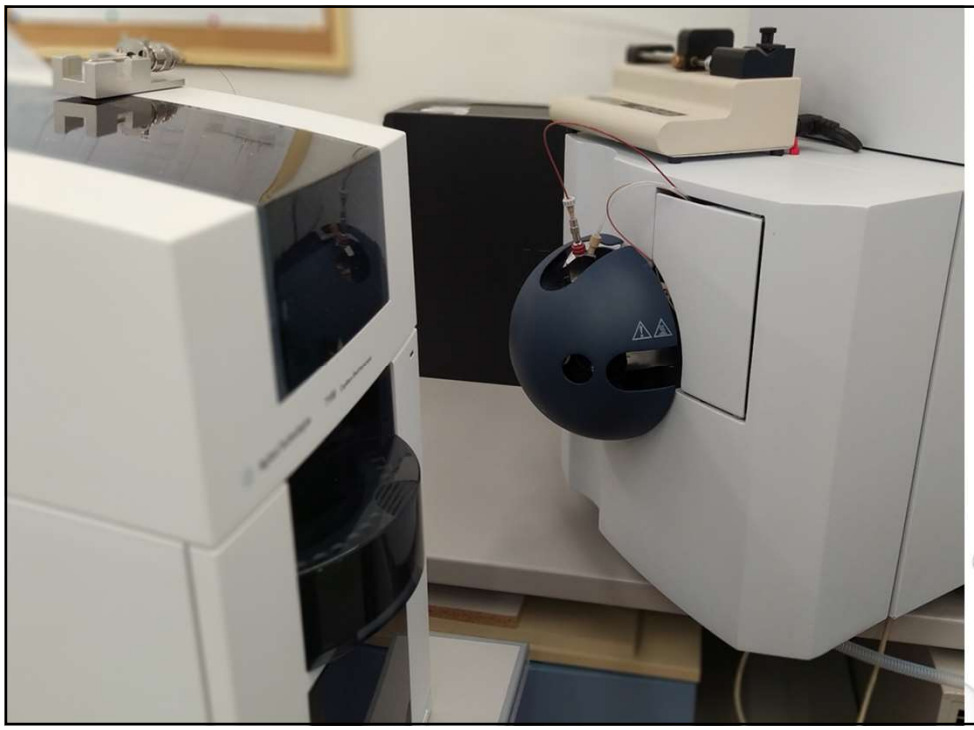


MASS

SPECTROMETRY

ANALYTICAL MASS SPECTROMETRY

1

AMBIENT IONIZATION TECHNIQUES

- ionization techniques working outside the mass spectrometer;
- objects of unusual shape and size can also be analyzed;
- enable direct sample analysis with minimal sample preparation;
- are usable as an ion source for most mass analyzers;
- they are soft and very gentle ionization techniques;
- they use the principles of common ionization techniques, but in an open space - ESI, CI, photoionization, etc.
- can also be used for mass spectrometric imaging;

2

AP-TD/SI	atmospheric pressure thermal desorption-secondary ionization
BADCI	beta electron-assisted direct chemical ionization
DAPCI	desorption atmospheric pressure chemical ionization
DAPPI	desorption atmospheric pressure photo-ionization
DART	direct analysis in real-time
DBDI	dielectric barrier discharge ionization
DCBI	desorption corona beam ionization
DEMI	desorption electrospray/metastable-induced ionization
DESI	desorption electrospray ionization
DICE	desorption ionization by charge exchange
EASI	easy ambient sonic-spray ionization
ELDI	electrospray-assisted laser desorption ionization
FAPA	flowing atmospheric pressure afterglow
IR-LAMICI	infrared laser ablation metastable-induced chemical ionization
LADESI	laser-assisted desorption electrospray ionization
LAESI	laser ablation electrospray ionization mass spectrometry
LDESI	laser desorption electrospray ionization
LESA	liquid extraction surface analysis
LIAD-ESI	laser-induced acoustic desorption-electrospray ionization
LMJ-SSP	liquid micro junction-surface sampling probe
LTP	low-temperature plasma probe
MALDESI	matrix-assisted laser desorption electrospray ionization
ND-EESI	neutral desorption extractive electrospray ionization
PESI	probe electrospray ionization
RADIO	radio-frequency acoustic desorption and ionization
REIMS	rapid evaporative ionization mass spectrometry
SwiFerr	switched ferroelectric plasma ionizer

3

AMBIENT IONIZATION TECHNIQUES (AI)

- Ionization outside the mass spectrometer - does not require sample pretreatment;
- rapid analysis from the surface of a solid sample at atmospheric pressure;
- 30 ambient ionization techniques (modifications);

Advantages:

- simplicity of analysis;
- high sample throughput;
- wide application area (analysis of explosives, drugs, forensic analysis...);

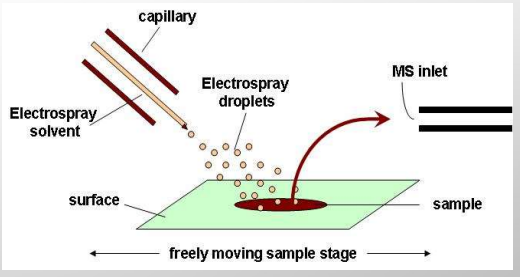
- the possibility of displaying the distribution of substances on the surface;
- combination with planar separation techniques (TLC);

4

AMBIENT IONIZATION TECHNIQUES (AI)

DESI (desorption electrospray ionization)

- extraction of the analyte from the surface using charged droplets
- Entry of secondary droplets („droplet pick-up“);
- Ions are created similarly to ESI, they create $[M+H]^+$, $[M+Na]^+$, $[M-H]^-$, $[M+Cl]^-$



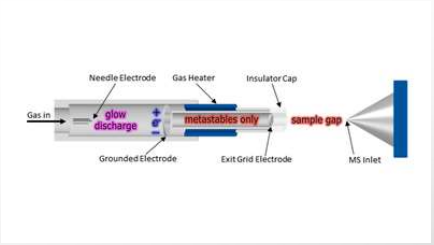
Optimization:

pressure and nebulizer geometry, voltage, flow, additives, surface material (glass, paper, polymer...)

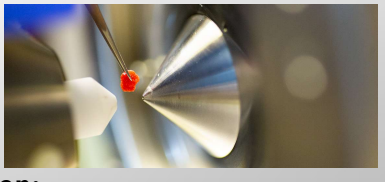
5

AMBIENT IONIZATION TECHNIQUES (AI)

DART– (direct analysis in real time)



- The sample is placed in the space before the MS;
- gas (He, N, Ne) is supplied to the ion source;
- discharge chamber - glowing discharge;
- ionization and formation of uncharged metastable gas particles;
- deflection of charged particles;
Only uncharged metastable particles interact – radicals;
Alternatively, interaction with H_2O and subsequent protonation;



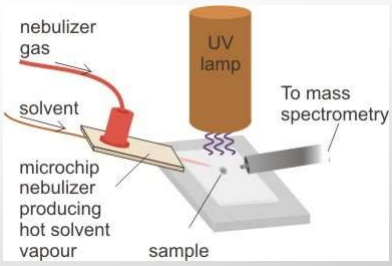
6

AMBIENT IONIZATION TECHNIQUES (AI)

DAPPI – (desorption atmospheric pressure photo-ionization)

A heated aerosol consisting of a solvent and a nebulizing gas is directed at the examined surface.

Desorption of the analytes will occur, which are subsequently photoionized by a UV lamp in the gas phase.



7

MATRIX-ASSISTED LASER DESOPTION/IONIZATION (MALDI)

- evolved from LD (*Anal. Chem 1978, 50, 985*), for the analysis of biopolymers;
- the stormy development of biochemistry in recent years (Nobel Prize in Chemistry 2002 for the invention of ESI and MALDI);
- (*Rapid Commun. Mass Spectrom. 1988, 2, 151*)

- sample mixed with matrix, then evaporated; a short laser pulse is absorbed by the matrix, the absorbed energy is transferred and ionization and desorption of sample ions occur;
- pulse ionization technique - basically in connection with a TOF analyzer;
- nitrogen **UV** lasers (3 ns pulse, UV 337 nm), **IR** lasers (CO2) are more expensive and less used (pulse 6-200ns);
- choice of matrix: aromatic carboxylic acids (dihydroxybenzoic acid, chlorsalicylic acid, cinnamic acid, etc.);
- delayed extraction - extracts ions up to about 10 - 100 ns after the application of the laser pulse, which equalizes their energies and thereby increases the resolution;
- biomolecules up to hundreds of thousands of Da can be ionized;
- the most frequently observed ions $[M+H]^+$, $[M+2H]^{2+}$, sometimes also $[M+3H]^{3+}$, $[M+Na]^+$, etc.

8

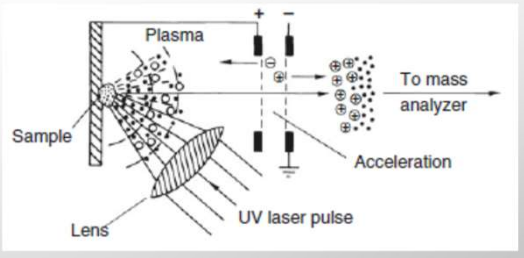
PRINCIPAL ARTICLES ON THE MALDI PRINCIPLE

- Michael Karas, Doris Bachmann and Franz Hillenkamp, *Anal. Chem.* ***1985****, 57, 2935-2939*
- Ronald C. Bavis and Brian Chait, *Rapid Com. In Mass Spectr. Vol.3, No. 7,* ***1989***
- Michael Karas, Matthias Glückmann and Jürgen Schäfer, *J. Mass Spectr. 35, 1-12, (****2000****)*
- Renato Zenobi and Richard Knochenmuss, *Mass Spectrometry Reviews,* ***1998****, 17, 337.366*

9

MALDI ANALYSIS PROCEDURE:

- 1.) Mixing the sample with the matrix in a suitable ratio (it is also possible separately);
- 2.) Applying a sub µl aliquot of the mixture to the target plate;
- 3.) Solvent evaporation and co-crystallization;
- 4.) Placing the target plate in the spectrometer;
- 5.) Application of laser pulse and ion generation;
- 6.) Ion analysis and data collection;



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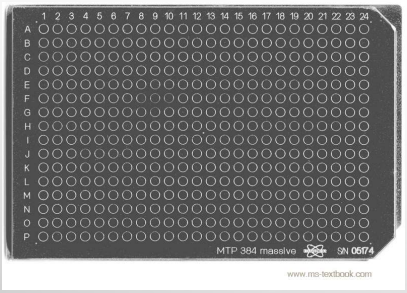
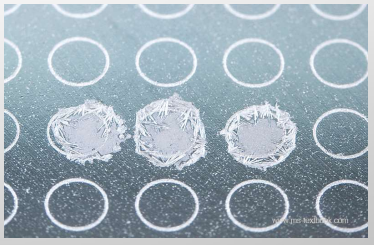
MALDI PLATE





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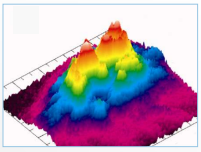
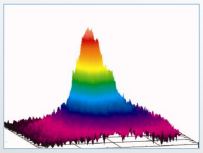
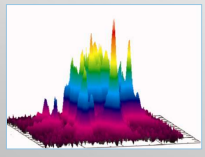
THE MOST COMMON TYPES OF LASERS:



Nitrogen laser:
pro: well structured energy profile
contra: slow (maximum 50Hz)



Nd:YAG laser:
pro: fast (up to 1000Hz)
contra: Gaussian energy profile (non-structured)



Smartbeam/Smartbeam II (modified Nd:YAG laser):
pro: fast (up to 1000Hz)
pro: well structured energy profile

12

THE MOST COMMON TYPES OF MATRIX:

- **MALDI matrix requirements:**
- **- Absorption at the wavelength of the laser used;**
- **- Appropriate crystallization;**
- **- For positive acid ionization (increased concentration of protons);**
- **- Stable, non-reactive with analyte, not very volatile;**

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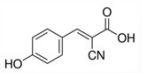
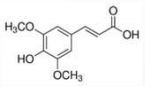
THE MOST COMMON TYPES OF MATRIX:

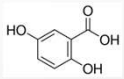
- **Peptides:** 4-Hydroxy-a-cyanocinnamic acid (**HCCA**)



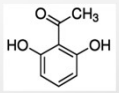
- **Proteins:** 3,5-Dimethoxy-4-hydroxycinnamic (Sinapinic) acid (**SA**)



2,5-Dihydroxy benzoic acid (**DHB**)

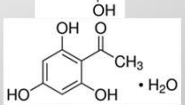


Dihydroxyacetophenone (**DHAP**)

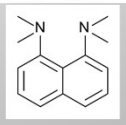


- **Glycans:** **DHB**
- **Nucleic acids:** 3-Hydroxypicolinic acid (**HPA**)

Trihydroxyacetophenone (**THAP**)



1,8-Bis(dimethylamino)naphthalene (Proton Sponge)



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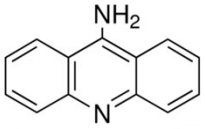
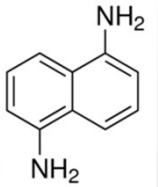
THE MOST COMMON TYPES OF MATRIX FOR **NEGATE IONS**

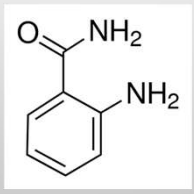
- **9-AMINOACRIDINE**



1,5-Diaminonaphthalene



Anthranilamide



15

THERMOSPRAY(TSI)

- designed for HPLC/MS connection;
- very gentle ionization technique (fragment ions are absent);
- the pressure in the ion source for TSI is higher than EI, but not API
- common mobile phases and flow rates (up to 1 ml/min);
- for RP-HPLC the necessary addition of electrolyte (ammonium acetate);
- the water content of the mobile phase must be at least 10%;

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- **a)** the metal capillary is heated to a temperature of T = 150 - 300C, a supersonic stream;
- **b)** small droplets carry an electrostatic charge on their surface, density of which gradually increases with further evaporation of the solvent;
- **c) Coulombic explosion;**
- **d)** the process of Coulombic explosions and evaporation is repeated - **"ion evaporation".**

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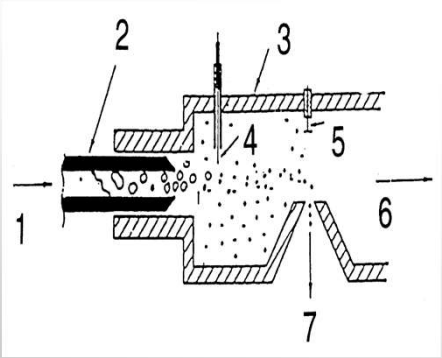
TSI



- **1/ output from HPLC;**
- **2/ heated capillary;**
- **3/ heated block;**
- **4/ discharge electrode (or source of accelerated e-);**
- **5/ repulsion electrode;**
- **6/ vacuum pumps;**

- the temperature of the heated capillary depends on the flow rate of the mobile phase and the solvents used;
- in general, TSI is not very suitable for low-polar substances;

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ATMOSPHERIC PRESSURE IONIZATION (API)

- a complete breakthrough in solving the HPLC/MS connection.
- an analytical technique of great importance for the structural analysis;
- identification of reaction products and impurities, trace analysis and, new possibilities in the field of biochemistry;
- **even electrons ions EE are formed almost exclusively !!!**
- Formation of molecular adducts (mainly ESI): $[M+H]^+$, $[M+Na]^+$, $[M+K]^+$, $[M-H]^-$, $[M+Cl]^-$, sometimes also adducts with a mobile phase molecule of the type $[M+methanol+H]^+$, $[M+acetonitril+H]^+$ or molecular adducts $[2M+H]^+$, $[3M+H]^+$
- Atmospheric photo-ionization (APPI) – suitable for compounds with very little polarity (e.g. polyaromatics);

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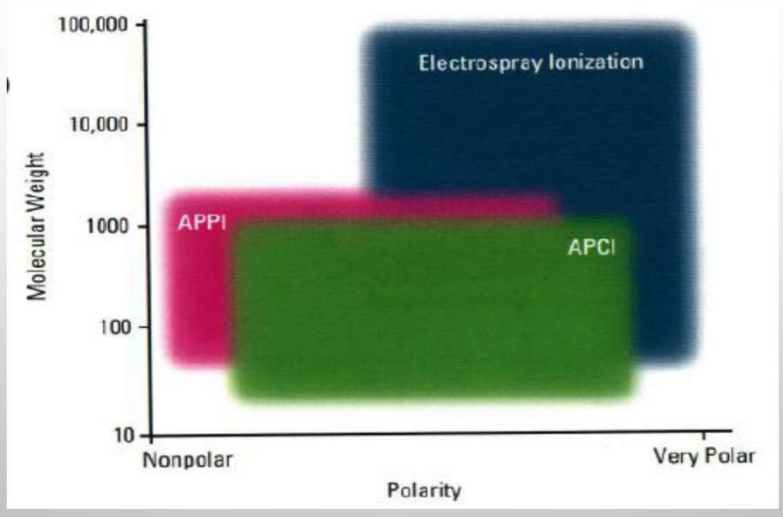
APPLICATION AREA OF API METHODS



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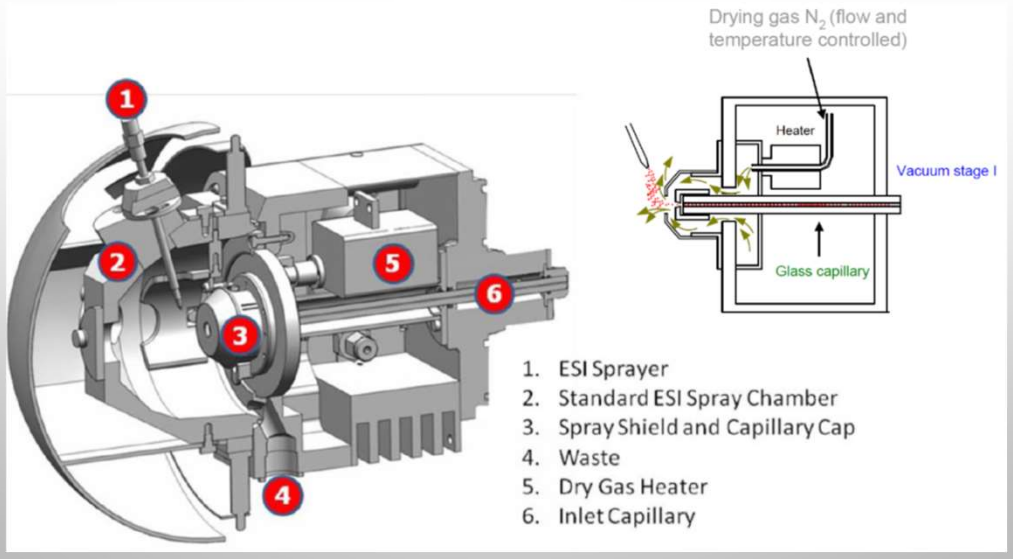
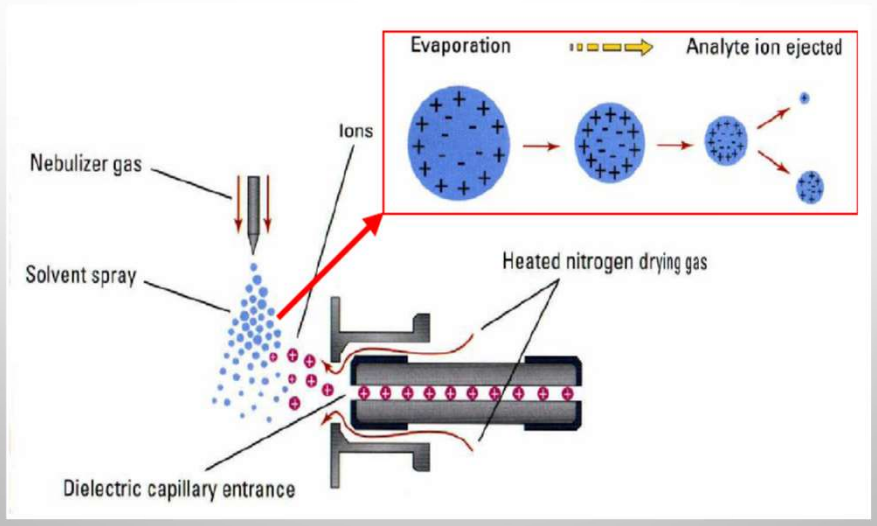
ELECTROSPRAY IONIZATION (ESI)



1. ESI Sprayer
2. Standard ESI Spray Chamber
3. Spray Shield and Capillary Cap
4. Waste
5. Dry Gas Heater
6. Inlet Capillary

ELECTROSPRAY IONIZATION (ESI)



ATMOSPHERIC PRESSURE CHEMICAL IONIZATION (APCI)

- Even electrone ions **EE** are formed almost exclusively, similar to ESI;
- compared to ESI, the intensity of adducts tends to be lower;
- relatively gentle ionization technique;
- fragment ions can be promoted by collision induced dissociation (CID);
- in-source CID or MS/MS setup;
- the possibility of a higher flow rate ml/min;

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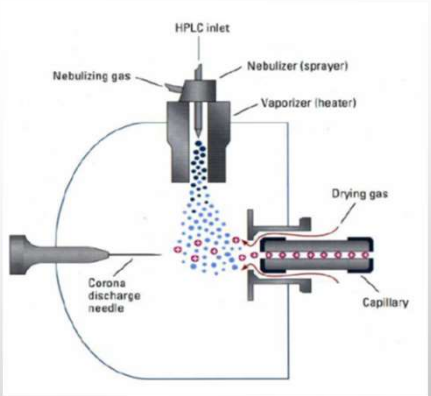
PRINCIPLE APCI

- output from HPLC (0.2 - 2 ml/min);
- a high voltage (3-4 kV) is applied to the discharge electrode ("discharge needle"),
- subsequently, the analyte molecules are ionized by **ion-molecular reactions** of the reaction gas (=ionized molecules of the mobile phase).
- counterflow of drying gas (nitrogen) is used to break up any non-covalent clusters and associates.



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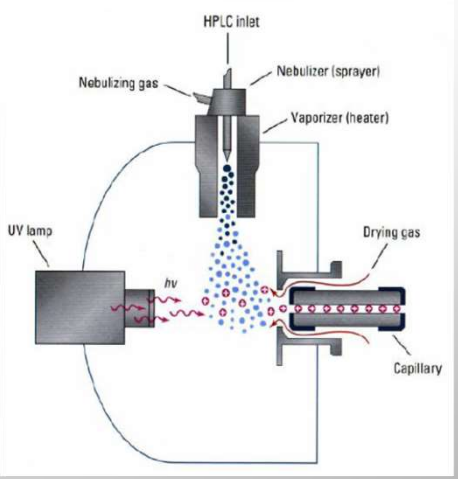
ATMOSPHERIC PRESSURE PHOTO-IONIZATION (APPI)

- analogy of APCI, only instead of corona discharge, UV radiation is used for ionization;
- the source of UV radiation is a **krypton discharge lamp;**
- Unlike ESI and APCI, ions with an odd number of e- can often be formed;
- Use of a dopant (toluene, benzene, IE<10 eV) – ion-molecular reactions occur;



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TYPES OF MASS ANALYZERS

- **Part of device which is used to the separation of ions according to m/z**
- **There are five general types of analyzers according to the different physical principles of ion separation.**
- 1/ **Magnetic Sector** – ions of certain m/z have a unique path radius in the magnetic field;
- 2/ **Quadrupole** - different stability of ion oscillations in a two- or three-dimensional combination of DC and RF voltages;
- 3/ **Time of Flight** (TOF) – sepatration of ions by time (without the use of an electric or magnetic field);
- 4/ **Ion Cyclotron Resonance** (ICR) The ions are in the magnetic field trapped into orbit inside. Different absorption of energy occurs during the cycloidal movement of ions;
- 5/ **Ion mobility** (IMS) separation ionized molecules present in the gas phase based on the mobility of the molecules in a carrier buffer gas;

26

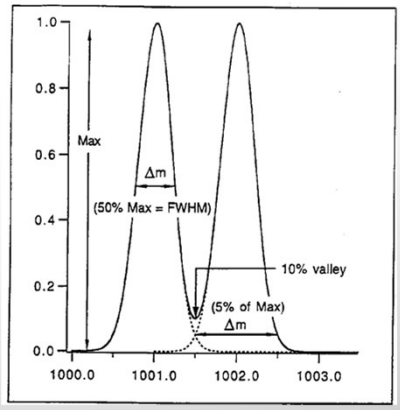
RESOLVING POWER

- **a) basic definition of resolving power, RP:** the peaks are sufficiently separated when the valley is 10%;

  $RP = m_1 / (m_1 - m_2)$

- **b) alternative definition** ratio of the mass to the Δm **(FWHM = full width at half maximum);**

  $RP = m / \Delta m$



Resolution (**R**) is the inverse of the value of **RP;**
R = 1 / RP
gives the relative difference of two ions that can still be distinguished in ppm;

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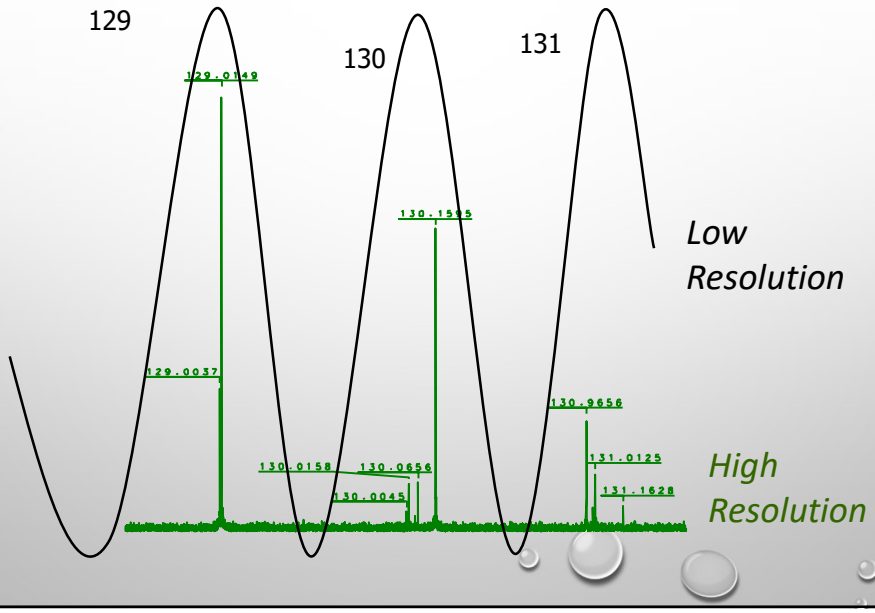
High Resolution vs. Low Resolution



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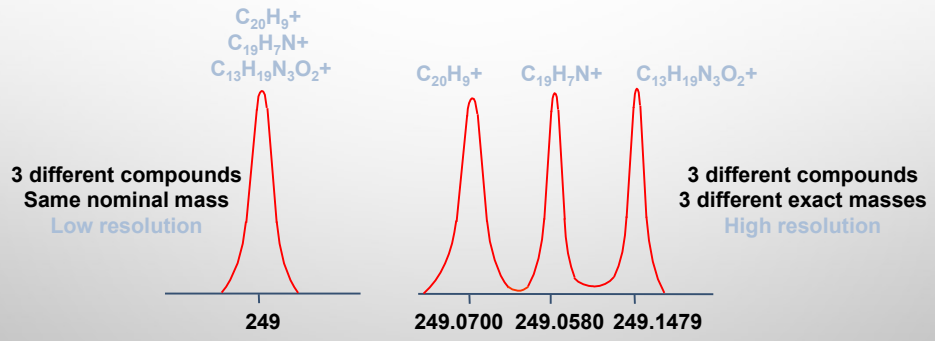
Resolution



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Mass Analyzers

	Resolving Power	Mass Accuracy
•Ion Cyclotron (FT-ICR-MS)	10 000 000	<1ppm
•Time of Flight (TOF)	40,000	3-10ppm
•Magnetic Sector	100 000	2-5ppm
•Quadrupole Ion Trap	1,000	n/a
•Quadrupole	1,000	n/a

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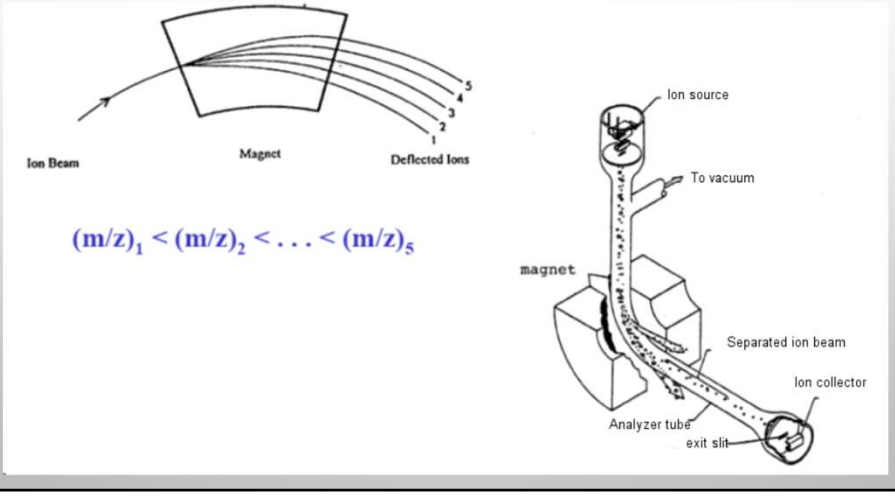
MAGNETIC SECTOR

principle: when passing through a magnetic field, the path of ions with a lower m/z value will be more curved; (the paths of heavier ions do not curve as much due to the greater centrifugal force of the heavier ion);



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PHYSICAL DESCRIPTION

- positive ions with a certain m/z value accelerated by a negative potential **V** enter a magnetic field with a magnetic induction **B,** resulting in the curvature of the movement of the ions on a trajectory of radius **r;**
- ions receive kinetic energy: $E_k = z.V = 1/2\ m.v^2$
- Magnetic field - centripetal force (Lorentz) **B.z.v**
  must be in balance with centrifugal force $m.v^2/r$
- $B.z.v = m.v^2/r$

- **basic equation of Magnetic Sector Mass spectrometer**
- $m/z = B^2.\ r^2/\ 2.V$

- Magnetig or potetntial scanning

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DOUBLE FOCUS MASS SPECTROMETER

- in addition to the magnetic focusing of the ions, there is also an electrical (electrostatic) focusing of the ions, resulting in a significant increase in the maximum RP;
- **principle:** in the electric field, the path of the ions will be curved depending on their E_k and regardless of the m/z value;
- to achieve higher resolution, we must energetically unite the ions;

33

PHYSICAL DESCRIPTION

- in an electrical sector, the **centripetal** electric force z.E is in balance with the **centrifugal** force $m.v^2/r$;
- **$z.E = m.v^2/r$**
- Kinetic energy of ions: $E_k = z.V = 1/2\ m.v^2$
- the equation for the radius of curvature of the trajectory in the electric field: **$r = 2.V/E$**
- **focus of ions in el. fields do not depend on the m/z ratio !!!**
- It serves to obtain a monoenergetic beam of ions;
- by combining magnetic (B) and electric (E) ion focusing we can achieve RP up to 100000;

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LINEAR QUADRUPOLE

- **principle**: the ion is brought to the center of the quadrupole axis and begins to oscillate; only the selected ion will pass through the quadrupole;
- scanning – **mass filter**
- **Physical destcription**: four metal cylindrically shaped rods 20 - 30 cm long;
- The pair of opposite rods are held at the same potential +DC, and –DC;
- RF is superimposed on all of them;

The advantages:
i) High transmission; ii) light-weighted and low price; iii) low ion acceleration voltages; iv) high scan speeds

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LINEAR QUADRUPOLE

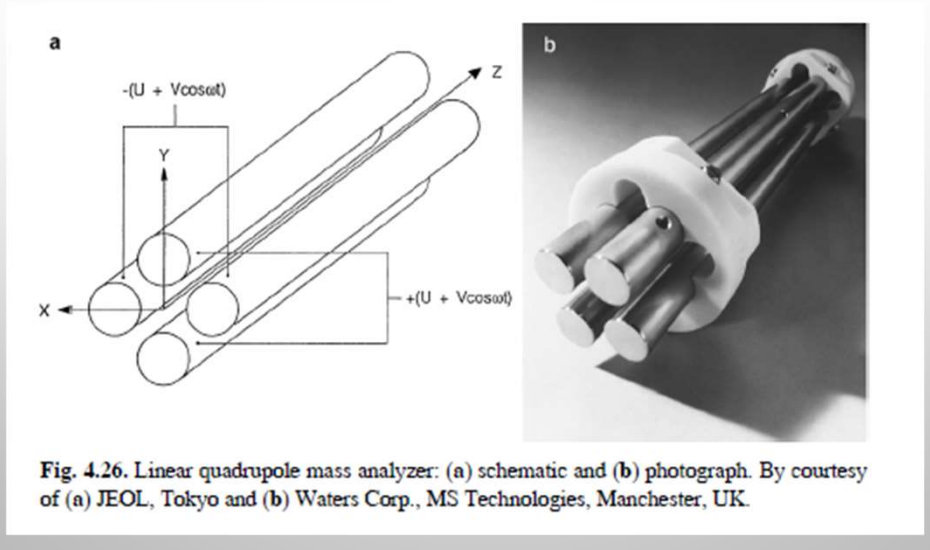

Fig. 4.26. Linear quadrupole mass analyzer: (a) schematic and (b) photograph. By courtesy of (a) JEOL, Tokyo and (b) Waters Corp., MS Technologies, Manchester, UK.

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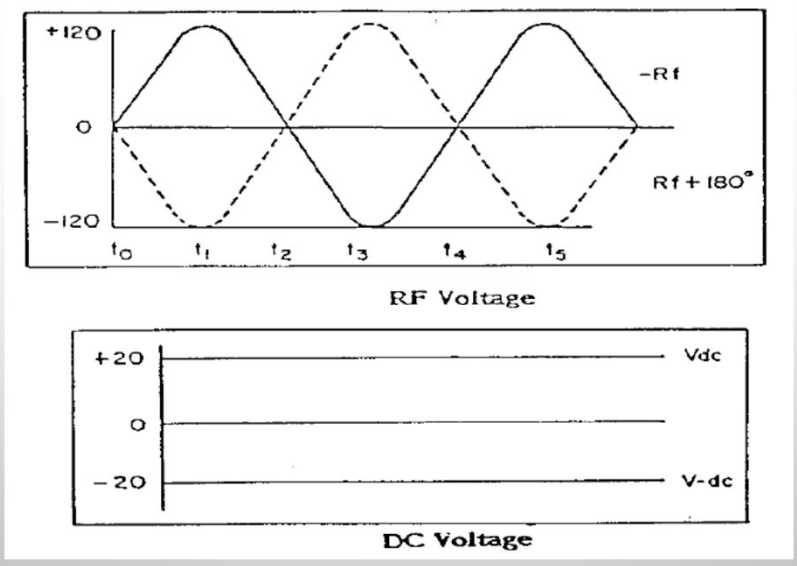

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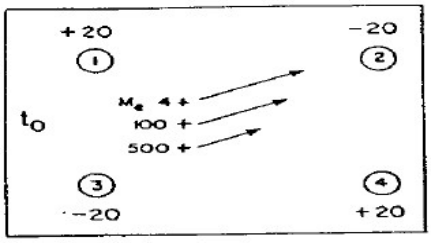
	rods 1 and 4	rods 2 and 3
to	+20	−20
t_1	+140	−140
t_2	+20	−20
t_3	−100	+100
t_4	+20	−20
t_5	+140	−140



Ion Positions at t_o

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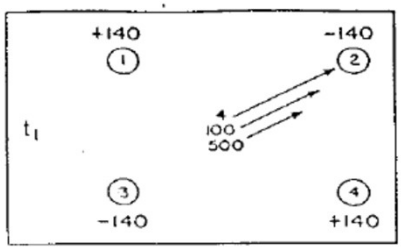
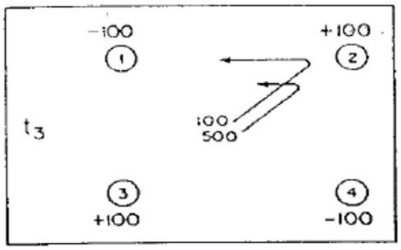


Ion Positions at t_1



Ion Positions at t_3

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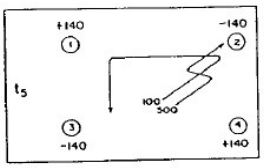
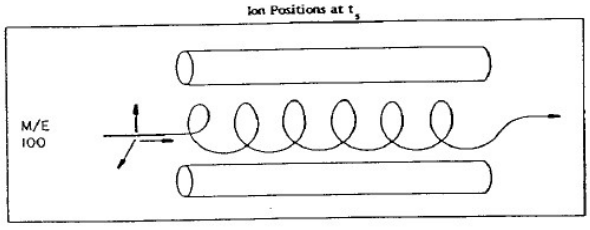


Figure 1.14 - Ion Trajectory for Bounded Ion

Ion Positions at t_5



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QUADRUPOLE ANALYSER

- hyperbolic shape – achieves a higher resolution;
- if we scan e.g. in the range of 1000 m/z, we only have 1/1000 of the total time to detect one m/z value, the rest of the time (i.e. 999/1000 of the time) the ions are captured on the quadrupole rods;
- reducing the mass range, we can increase the detection sensitivity!
- Selected Ion Monitoring, (SIM), can increase sensitivity 1000 times;
- for quantitative and ultra-trace analysis GC/MS a HPLC/MS;

THREE-DIMENSIONAL QUADRUPOLE ION TRAP

- **Principle:**
- The QIT consists of two hyperbolic electrodes serving as end caps along with a ring electrode that replaces two of the linear quadrupole rods;
- ions are pulsed into the trap, where they are captured and gradually ejected onto the detector according to their m/z;
- external ionization (mostly for ESI/APCI) or internal ionization inside the trap (EI/CI);

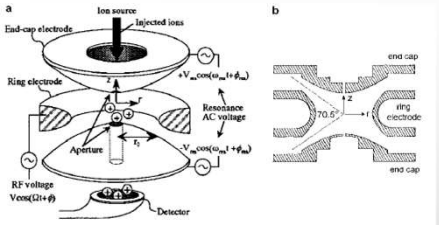

Fig. 4.43. A quadrupole ion trap. (a) QIT with external ion source (illustration stretched in z-direction) and (b) section in the rz-plane (in scale). (a) Reproduced from Ref. [132] by permission. © John Wiley & Sons, 2000.

Fig. 4.44. Electrodes of the Finnigan MAT ITS40 quadrupole ion trap. By courtesy of Thermo Electron (Bremen) GmbH.