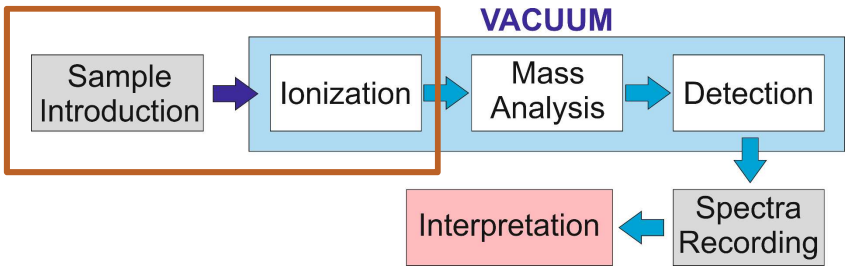
Mass spectrometry
Generation of ions

Literature: Jürgen H. Gross: Mass Spectrometry

Content:



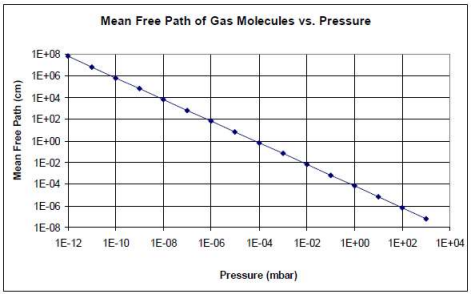
- What kind of chemicals can we analyze with MS?
- How can we introduce a sample to a mass spectrometer and how?
- What information can we expect from the analysis?

1 bar = 10^5 Pa
1 bar = 750.06 Torr
1 bar = 0.98682 Atm

Why do we need vacuum?

- Collisions perturb mass determination in MS methods
- Collisions can lead to reactions → modifications of ions

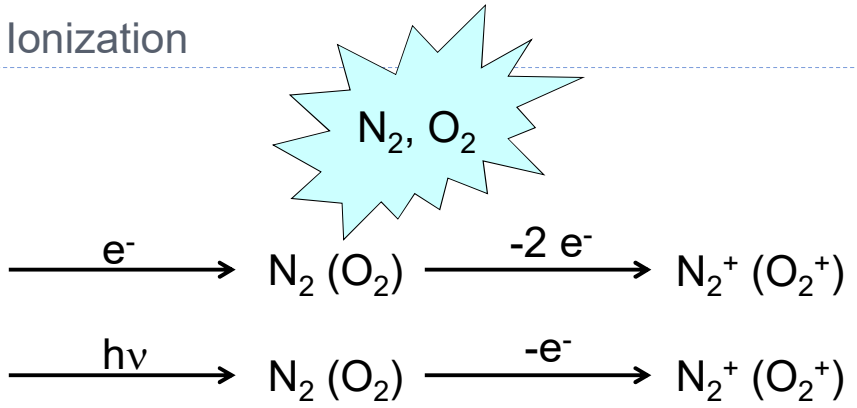
- λ = 0,66/P (λ in cm, P in Pa)
  - Mean Free Path
  - Typical ion paths
    - Quadrupole – 20 cm
    - TOF – 2 m
  - P = 10^{-4} mbar → $\lambda = 0{,}66$ m
  - P = 10^{-5} mbar → $\lambda = 6{,}6$ m
  - Typically 10times to 100times longer path then the length of the analyzer → decrease of collision probability to 10 %, resp. 1 %



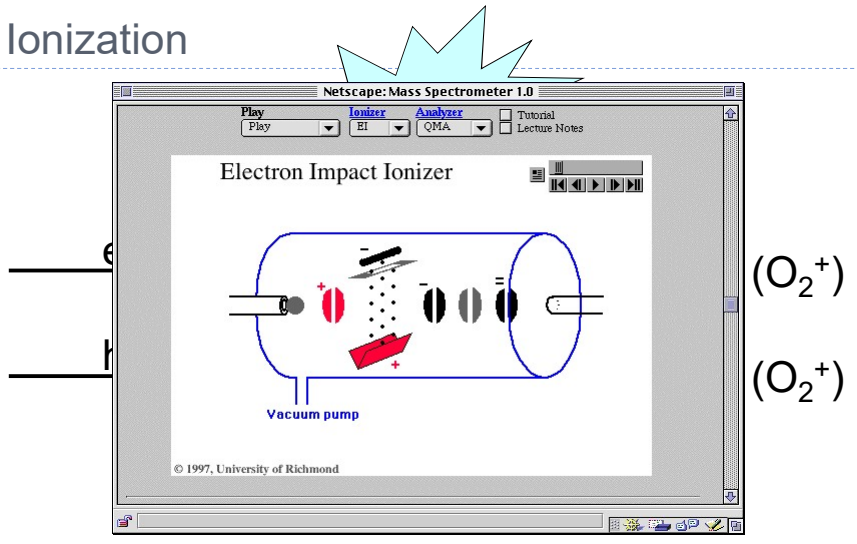
How do we bridge ambient conditions with low pressure environment?

- Classic electron ionization → ions are formed in “vacuum”
  - Gaseous, liquid, solid samples need to be introduced to an ion source with pressure of < 10^{-3} mbar
    - via a low pressure coupling
    - solid samples – via an evacuation chamber
- “Ambient” ionization methods – ions are formed before they are introduced to “vacuum” (ESI, API, MALDI)
  - Samples can be dissolved in a suitable solvent (ESI, API)
  - Samples can be solid (MALDI)

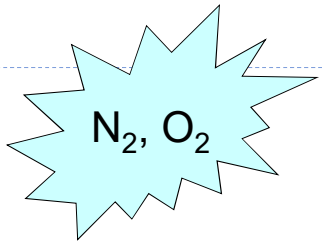
Ionization



Ionization



Ionization

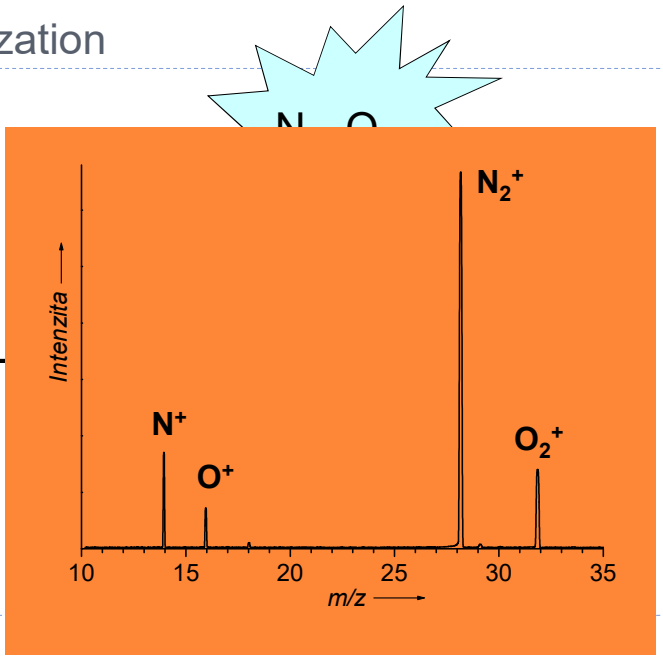


$$\xrightarrow{e^-} N_2\ (O_2) \xrightarrow{-2\ e^-} N_2^+\ (O_2^+)$$

$N_2^+ \rightarrow N^+$

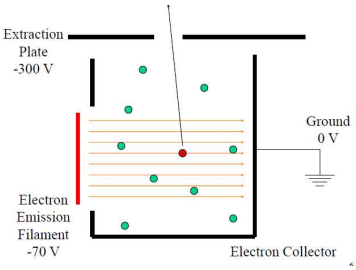
$(O_2^+) \rightarrow O^+$

Ionization



$(O_2^+) \rightarrow O^+$

Electron ionization



1. Electrons are emitted by a hot filament (Re, W)
2. Electrons are accelerated by a potential difference towards an anode
3. Electrons interact with gaseous molecules in the ion source

$$Ionization\ efficiency = \frac{number\ of\ the\ generated\ ions}{number\ of\ neutral\ molecules}$$

Ionization energy

Ionization energy of common chemicals up to ~ **15 eV**
Kinetic energy of electrons: **70 eV**

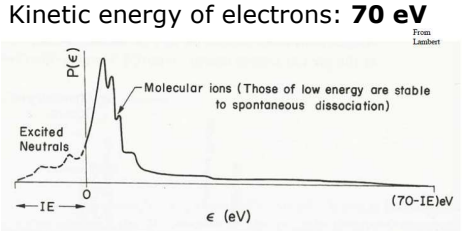
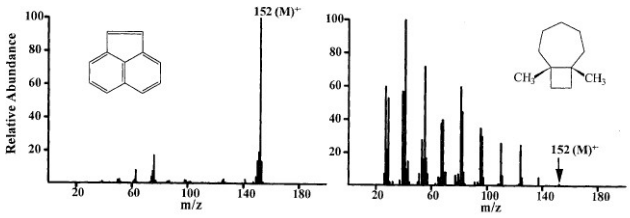


Figure 14-28 Stylized internal energy distribution $P(\varepsilon)$ resulting from impact by electrons of 70 eV kinetic energy on an organic molecule. The maximum internal energy that an ion can acquire is 70 eV, minus the ionization energy (IE). Most ions acquire small internal energies.

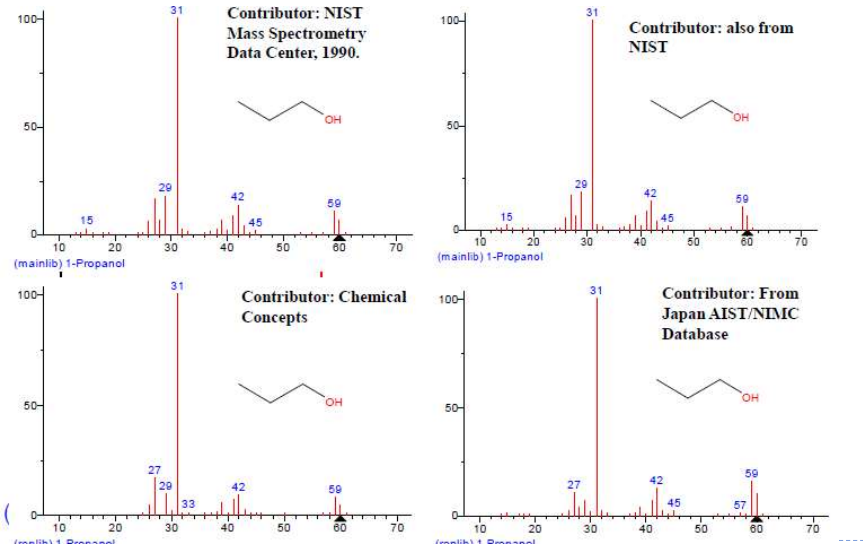
EI generates ions with large distribution of internal energy

~0 – 15 eV

→ many fragments in the EI mass spectra



Reproducibility of EI spectra



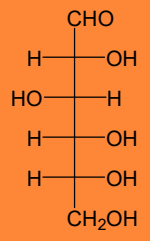
Advantages and disadvantages of EI

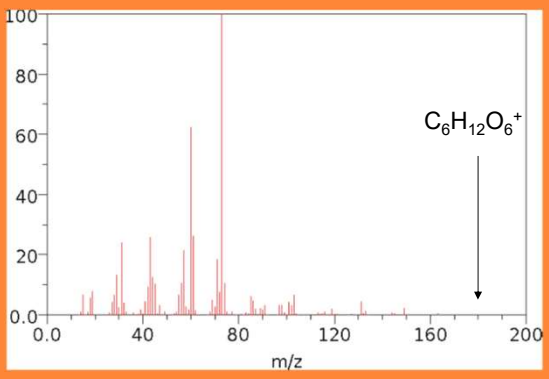
Advantages	Consequences
Reproducibility	Libraries of EI spectra → identification
Large fragmentation	We can deduce molecular structure
Large ionization efficiency	Sensitive method (1 molecule out of 1000 is ionized)
Disadvantages	**Consequences**
Only positive ions	Not suitable for all kinds of compounds
Radical cations	Rearrangements
Sample most be volatile	Works only for compounds with low molecular weight (~ 600 Da)
Ionization is not selective	All molecules present in the ion source contribute to the mass spectrum
Hard ionization method	Pronounced fragmentation sometimes precludes determination of molecular mass

Electron ionization – “hard” ionization

$C_6H_{12}O_6$

M = 180 g/mol



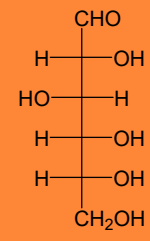


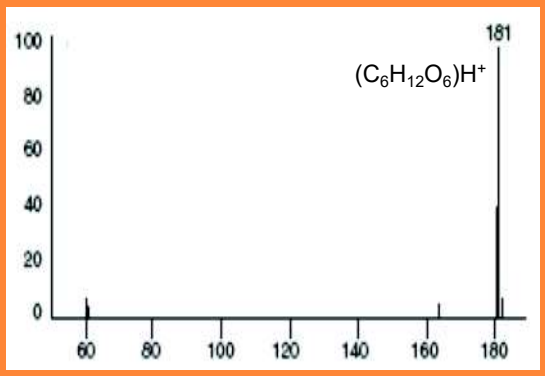
“Soft” ionization techniques

- chemical ionization
  - H_2O → H_3O^+ → protonation of the studied molecule

$C_6H_{12}O_6$

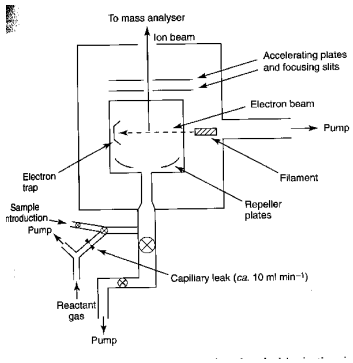
M = 180 g/mol





Chemical ionization (CI)

- 1966: Munson a Field
  - CI was discovered based on investigation of ion-molecule reactions
  - The method is based on reactions of analyte M with ions of reaction gas $R^{\pm}$



1. Similar to EI source
2. Larger pressure in the source
3. Sample (analyte) and reaction gas are introduced at the same time

CI reactions

General steps during CI

- Reaction gas R is ionized by electron ionization
  For example: $CH_4 \rightarrow CH_4^{+\bullet}$

  Large excess of R over M (<100 : 1) ensures preferential ionization of R

- The initially formed ions $R^{\pm}$ react with other molecules of R to form longer lived ions $R'^{\pm}$
  For example: $CH_4^{+\bullet} + CH_4 \rightarrow CH_5^+ + CH_3^{\bullet}$

  The reactions require collisions → we need a larger pressure in the source

- In collisions of $R'^{\pm}$ with analyte M, the analyte is ionized to $M_1^{\pm}$
  $R^{\pm} + M \rightarrow M_1^{\pm} + N_1$
  For example: $CH_5^+ + C_6H_{12}O_6 \rightarrow (C_6H_{12}O_6)H^+ + CH_4$

- Analyte ions can fragment
  $M_1^{\pm} \rightarrow M_2^{\pm} + N_2$ or $M_3^{\pm} + N_3$ or $M_4^{\pm} + N_4$

Chemical ionization

- Common reactions:

Proton transfer:	$RH^+ + M \rightarrow MH^+ + R$
Electron transfer:	$R^{+\bullet} + M \rightarrow M^{+\bullet} + R$
Electron capture:	$R + e \rightarrow R^-$
Adduct formation:	$RH^+ + M \rightarrow (M\text{-}RH)^+$
	$MH^+ + M \rightarrow (M\text{-}MH)^+$

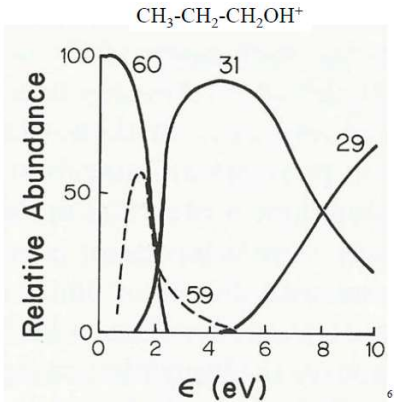
- Fragmentation upon CI depends on exothermicity of the given reaction:
  Proton transfer: $\Delta H = PA(R) - PA(M)$

  Note: Proton transfer occurs if PA(M) > PA(R)

- MS spectra are affected by selection of reaction gases → possible to obtain different information

Breakdown pattern of 1-propanol

- The energy transferred during chemical ionization is well defined



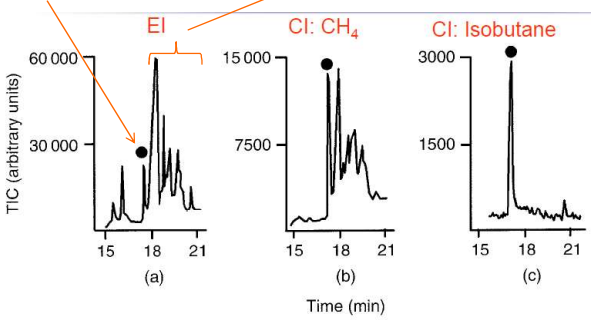
Selective detection

GC-MS

Problem: We analyse a mixture of C11-hydrocarbons with a small admixture of butylmethacrylate. We want to selectively detect butylmethacrylate, therefore we will take an advantage of its larger proton affinity compared to the pure hydrocarbons. The spectra show that using a reactant gas with a larger proton affinity (isobutane) will more selectively detect butylmethacrylate than using a reactant gas with a very low proton affinity (methane). Note, that electron ionization is a non-selective method.

butylmethacrylate

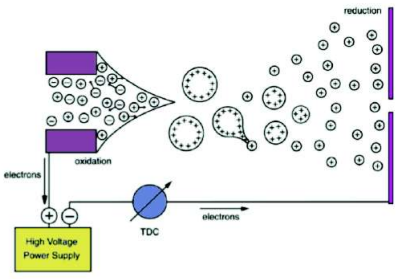
C11-hydrocarbons

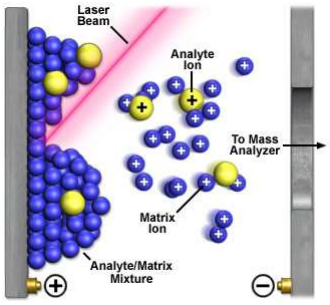


- Proton affinity of reaction gas determines what molecules can be protonated

Soft ionization – MS opens to biochemistry

- electrospray ionization
- MALDI

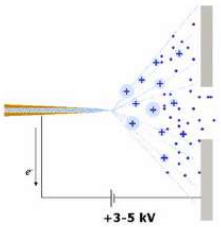


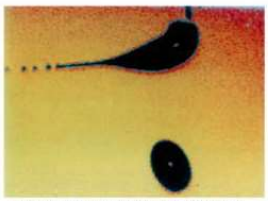


- https://www.youtube.com/watch?v=YA5DDt6bMlw&list=PL2uPQ1QXgo-7_qfDtjaGD4THx7JzTVEWP (watch 0:40 – 1:20)

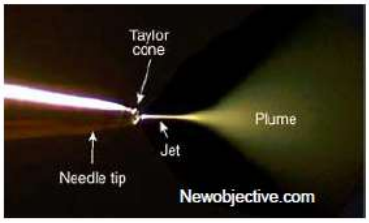
Electrospray ionization

- Atmospheric pressure ionization
- Ionization of large nonvolatile molecules (proteins) without fragmentation
- Connection to HPLC
- Mechanism
  - Solution infused by a capillary to the interface with a potential difference of 3 – 5 kV
  - Liquid stream → Taylor cone → strand → droplets
  - Evaporation of solvent from droplets increases charge density
  - Raylegh limit – repulsion force between cations is equal to the surface tension of the droplet → explosion (charge residue model or ion evaporaton model)
  - Coulombic explosions lead to generation of isolated ions (positive or negative)



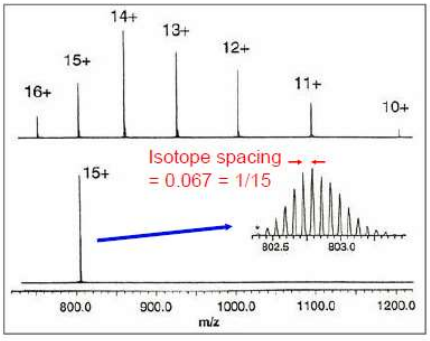


Gomez & Tang, *Phys Fluids*, 1994, 6:404–414



Characteristic ESI-MS spectrum

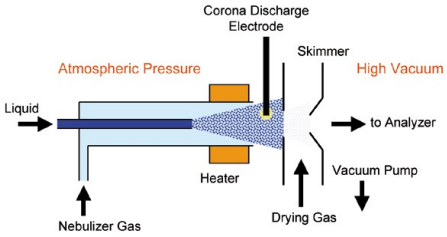
- Multiply charged ions
- Multiple charge → small *m/z* → advantageous for most of the mass analyzers
- *z* can be determined from the spacing of the peaks



ESI-MS of Cytochrome C, ~12,360 Da

From Fig 13-18 Lambert

APCI – Atmospheric pressure chemical ionization



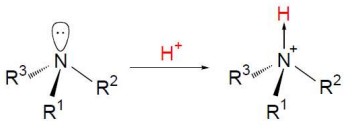
- Connection of MS a LC
- Heat and N_2 streem – drops desolvation
- Discharge ionizes solvent molecules (reaction gas)
- Not suitable for thermo-labile samples

What do we know about ionization of neutral molecules?

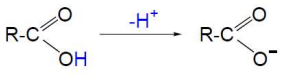
- Where and how are neutral molecules ionized?
  - In solution, in droplets, in the gas phase
  - By formation of adducts with Na^+, Li^+, NH_4^+ from salts, with H^+ from acids, with Cl^- from salts/solvent
  - Electrochemically (H^+ from water, radical cations)
  - Proton transfer reactions in the gas phase
- (Dis)similarity of chemistries in the gas phase and in solutions
  - Proton transfer in solution → pK_a
  - Proton transfer in the gas phase → PA (proton affinity)
  - They are not necessarily analogous

Chemistry during ESI

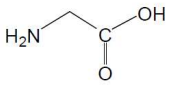
- ESI+ detects polar molecules containing O, N a S and some hydrocarbons such as isoprene, terpenes, aromatics as their protonated molecules MH^+



- ESI- detects acids (e.g. carboxylic acids, anorganic acids) and thiols as their deprotonated anions $[M-H]^-$



Glycine (bifunctional)



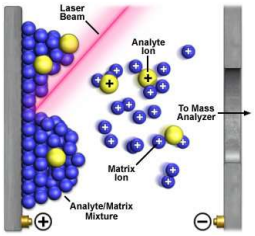
ESI in praxis

- ESI chemistry has many control parameters and unknown effects
- Understanding requires a good knowledge of organic chemistry and ion chemistry
- ESI is one of the fastest and most powerful ionization methods in mass spectrometry

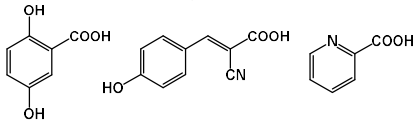
Analyte	ESI polarity	ESI reagent	ΔPA (vs. H_2O, R=CH_3)
carboxylic acid R-C(=O)-OH	- (or +)	methanol (or ACN + trace H_2O)	22.3 kcal/mol
ketones R-C(=O)-CH_3	+	methanol (or ACN + trace H_2O)	29.0 kcal/mol
aldehydes R-C(=O)-H	+	methanol (or ACN + H_2O) + HCOOH, LiCl, or NaCl	18.7 kcal/mol

cf. Lithium ion attachment MS (Selvin, Iwase and Fujii, Anal.Chem.**74**, (2002) 2053

Matrix assisted laser desorption ionization



- Analyte mixed with a crystalline matrix



- Dry mixture irradiated by a short, intense laser pulse at a wavelengths absorbed by the matrix (usually UV)
- Fast matrix heat-up → sublimation and expansion to the gas phase
- Ionization – proton transfer
- Usually singly charged ions, suitable for large molecules

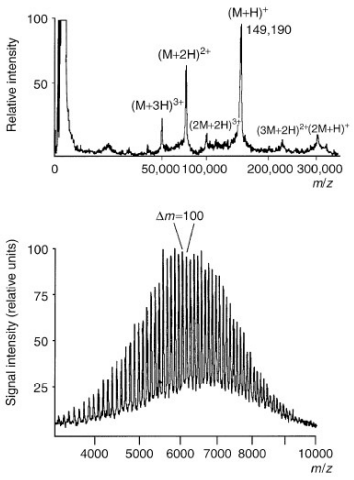
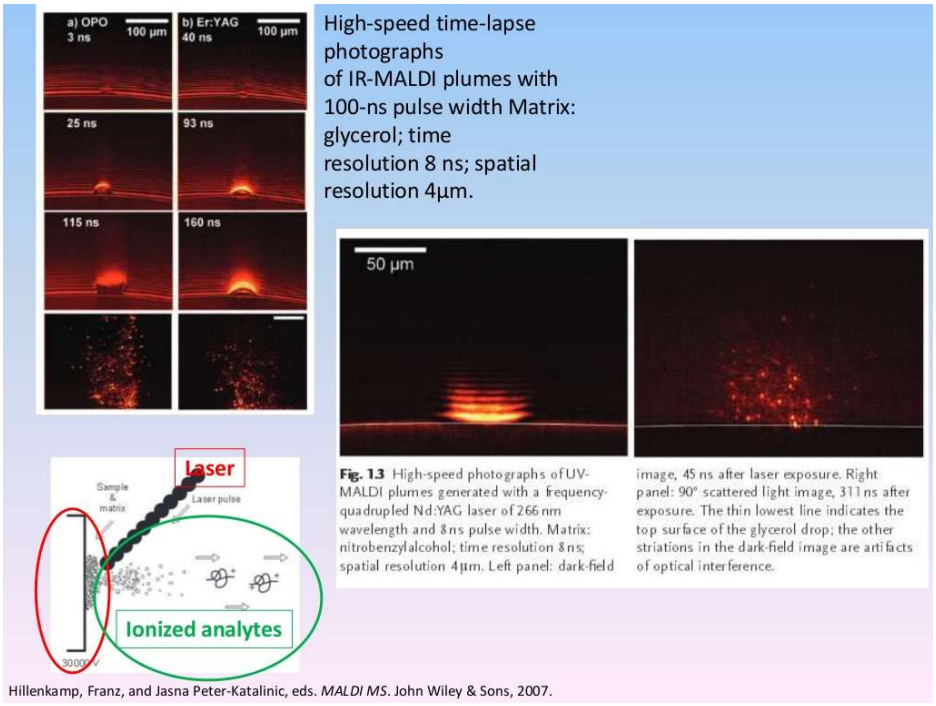


Figure 1.15
The MALDI spectra of a monoclonal antibody (*top*) and poly(methyl methoacrylate) of average mass 7100 Da (*bottom*) (Reproduced (modified) from Ref. 24 and from Finnigan MAT documentation, with permission)

Matrices

Matrix	Solvents	Wavelength (nm)	Analytes
2,5-dihydroxybenzoic acid	ACN, H_2O, CH_3OH, acetone, $CHCl_3$	337, 355, 266	Peptides, (oligo)nucleotides, oligosacharides
3,5-dimethoxy-4-hydroxycinnamic acid	ACN, H_2O, acetone, $CHCl_3$	337, 355, 266	Peptides, proteins, lipids
α-cyano-4-hydroxycinnamic acid (CHCA)	ACN, H_2O, EtOH, aceton	337, 355	Peptides, lipids, nucleotides
Picolinic acid (PA)	EtOH	266	oligonucleotides



Hillenkamp, Franz, and Jasna Peter-Katalinic, eds. *MALDI MS*. John Wiley & Sons, 2007.

Summary:

- What kind of chemicals can we analyze with MS?
  - gaseous, liquid, solid, solutions
- How can we introduce a sample to a mass spectrometer?

  1) introduce a sample directly into the mass spectrometer (pass to the low pressure range P < 10^{-3} mbar) – electron ionization/ chemical ionization
  - if the sample is solid, it must be volatile at P < 10^{-3} mbar

  2) introduce a sample by ambient ionization (the analyte is transformed to ions before entering the low pressure range) – electrospray ionization, atmospheric pressure ionization, MALDI
  - suitable even for non-volatile samples such as proteins
- What information can we expect from the analysis?
  - m/z ratio of the analyte (sometimes not available for unstable ions) and information about elemental composition (see video Interpretation_1)
  - indirect information about structure (e.g. fragmentation patterns in EI)