MALDI- TOF Mass Spectrometry

Solving practical problems

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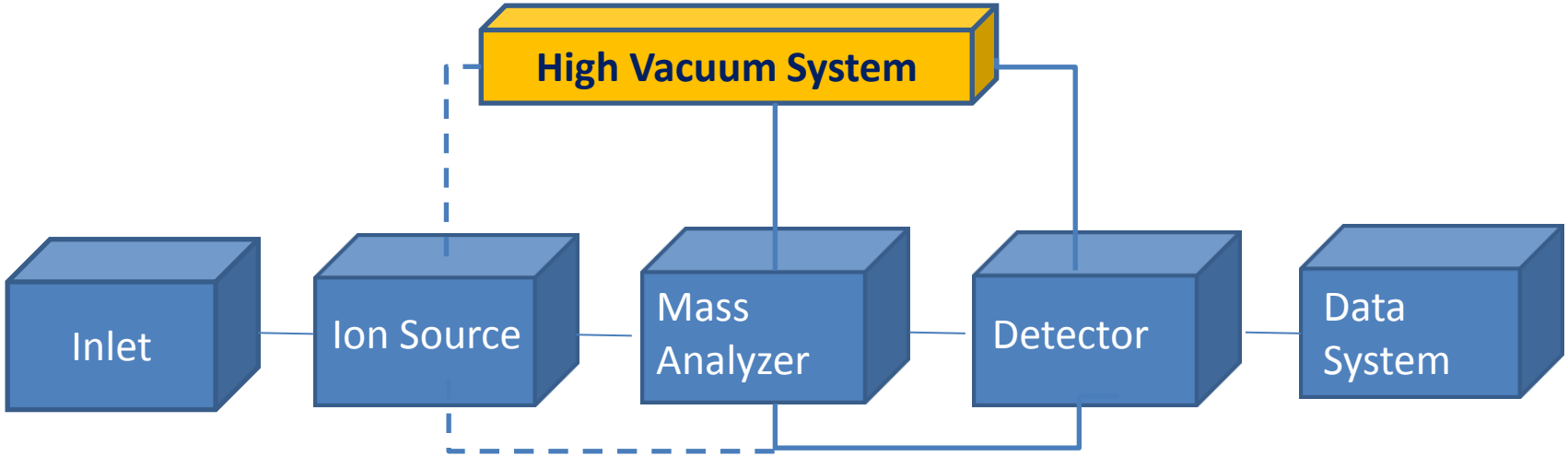
What does a mass spectrometer do?

- ✓ It measures mass better than any other technique.
- ✓ It can give information about chemical structures.

What are mass measurements good for?

- ✓ To identify, quantitate and verify.

Mass spectrometer



Sample introduction
HPLC,
Flow injection,
Sample plate

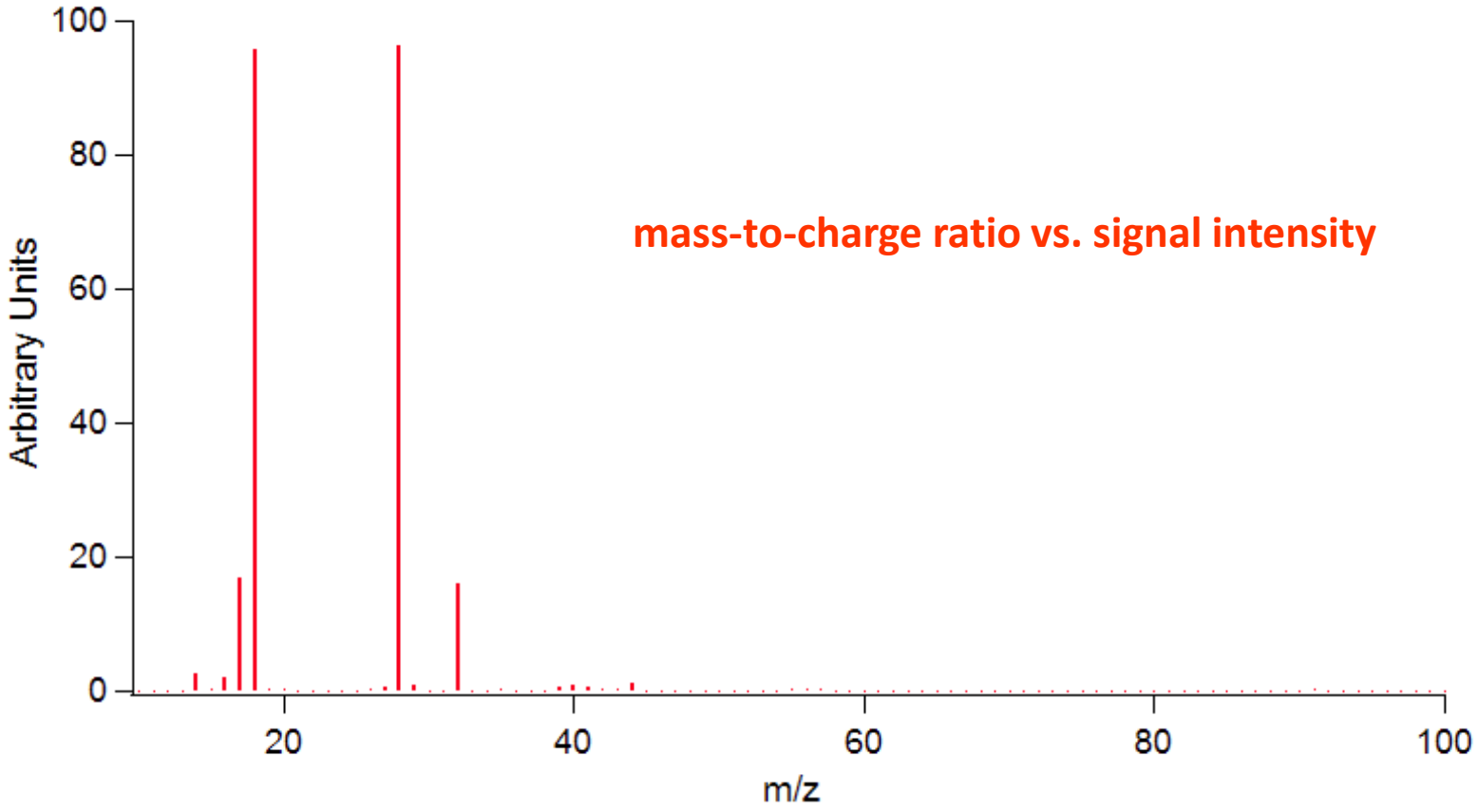
Ion formation
ESI
MALDI
EI
CI
…

Ions separation in accordance with they m/z
Time-of-flight
Quadruple
Ion trap
FT ICR
Double Sector
…

Ions detection
Electron multiplier
…

Mass spectrum generation
PC software

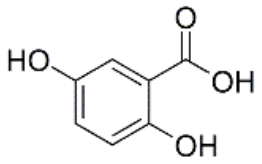
Mass spectrum



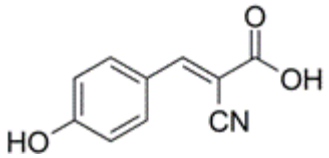
mass-to-charge ratio vs. signal intensity

Matrix Assisted Laser Desorption/Ionization (MALDI)

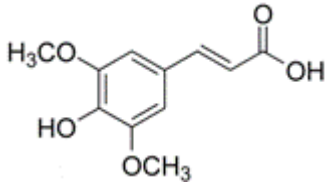
- ✓ Method where a **laser** (nitrogen gas UV laser, 337nm) is used to generate **ions** from neutral analyte molecules
- ✓ Analyte is embedded in to crystal **matrix**
- ✓ The presence of matrix causes the molecules to ionize <u>instead of decomposing.</u>



2,5-dihydroxybenoic acid

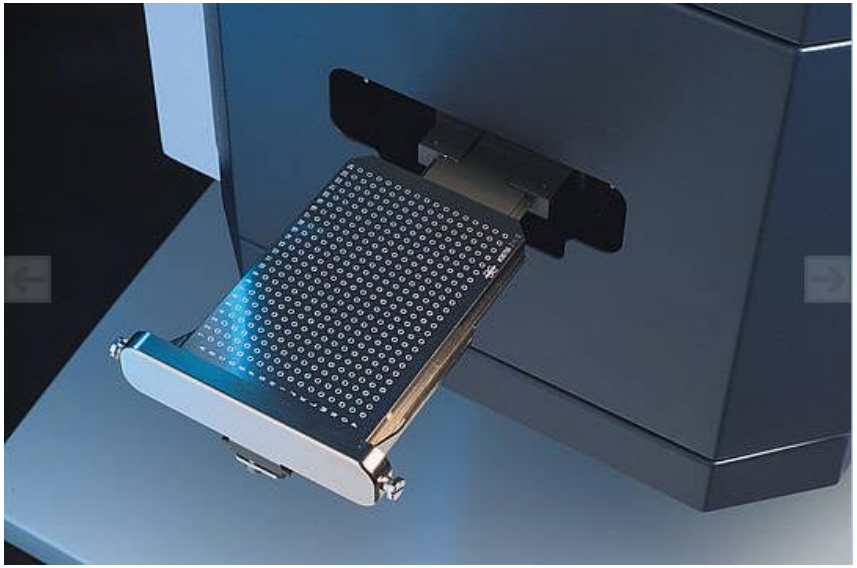


α-cyano-4-hydroxycinnamic acid

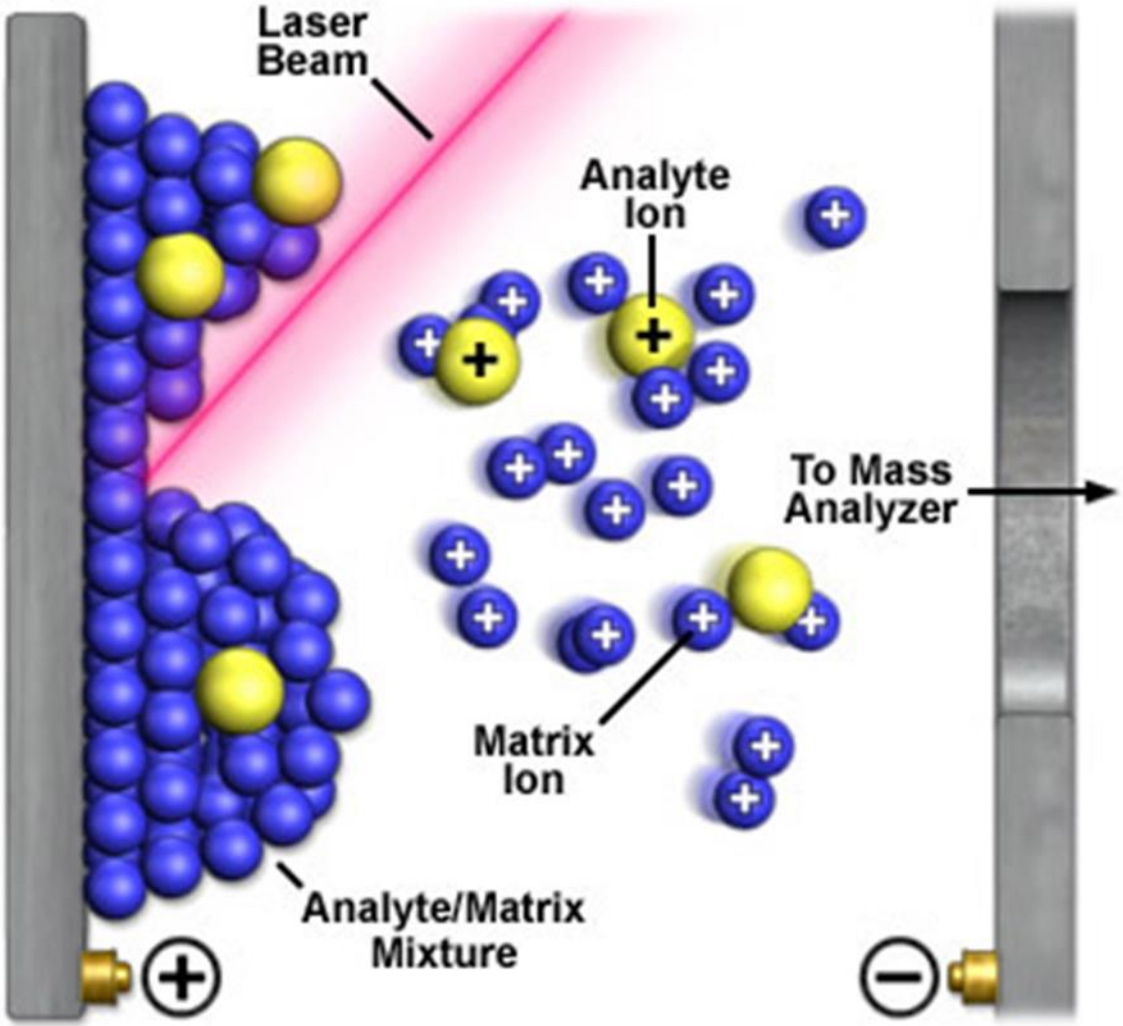


sinapinic acid

Bruker Autoflex II MALDI TOF



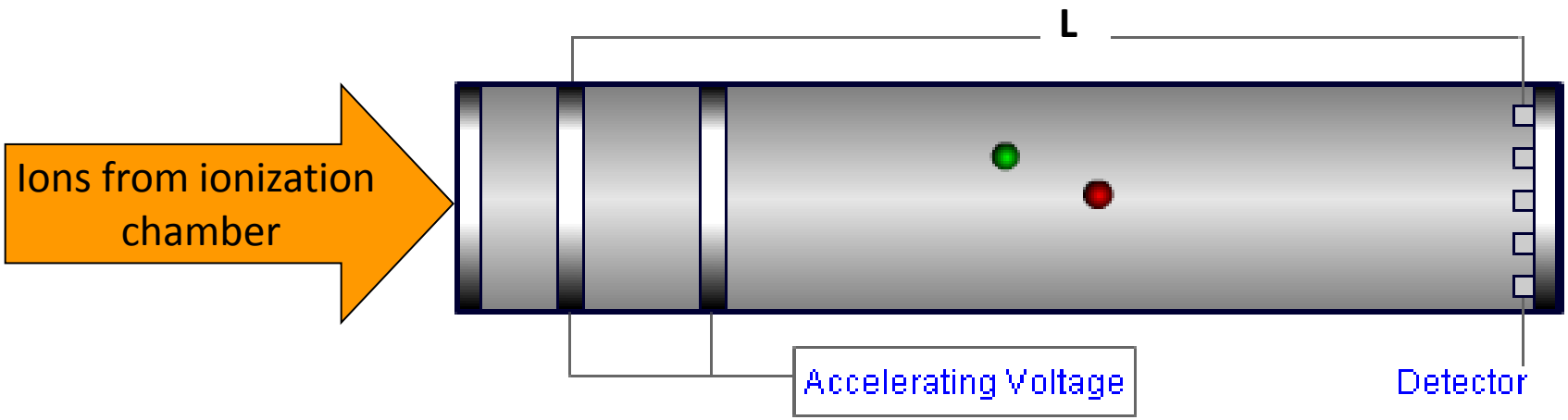
MALDI Ionization Mechanism



1. Sample (A) is mixed with excess matrix (M) and dried on a MALDI plate.
2. Laser flash ionizes matrix molecules.
3. Sample molecules are ionized by proton transfer from matrix:

   $MH^+ + A \rightarrow M + AH^+$.

Time – of – Flight mass analyzer



The mass-to-charge ratio of an ion is proportional to the square of its drift time.

$$\frac{m}{z} = \frac{2t^2K}{L^2}$$

t = drift time
L = drift length
m = mass
K = kinetic energy of ion
z = number of charges on ion

Applications of MALDI-TOF

- ✓ Peptides and proteins
- ✓ Synthetic polymers
- ✓ Oligonucleotides
- ✓ Oligosaccharides
- ✓ Lipids
- ✓ …

What about small molecules ?

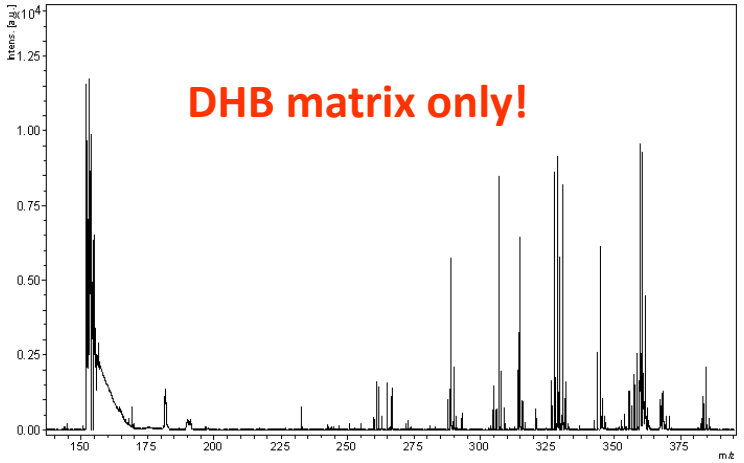
MALDI Advantages

- Soft Ionization technique (no molecule fragmentation)
- High molecular weight analyte can be ionized
- Molecule need not be volatile
- Sub-picomole sensitivity easy to obtain

MALDI Disadvantages

- MALDI matrix cluster ions obscure low m/z species (<600)
- Signal reproducibility is low
- Coupling MALDI with chromatography can be difficult

PROBLEM



Low mass range contamination

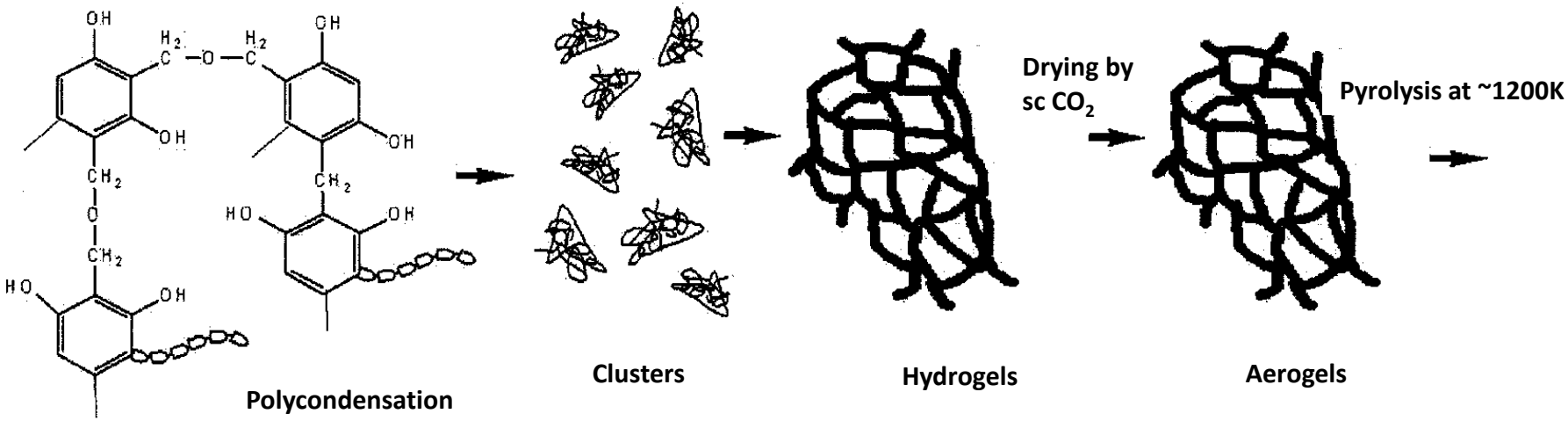
SOLUTION

New types of matrixes or matrix free surface (SALDI)

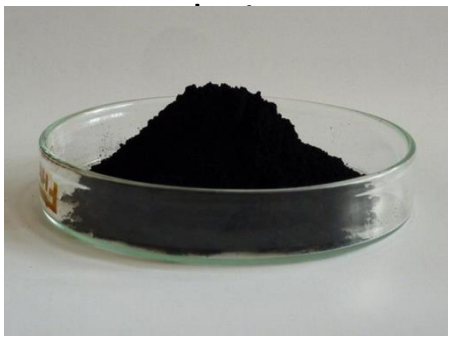
SALDI – ~~matrix~~ Surface-Assisted Laser Desorption/Ionization

- ✓ Carbon nanotubes
- ✓ Silicon nanoparticles
- ✓ Graphite
- ✓ Metal oxide particles (ZnO, TiO_2, Fe_2O_3 etc.)
- ✓ Metal nanoparticles (Au, Ag, Mo, Zn etc.)
- ✓ POWDERED CARBON AEROGELS (particle size 10-30nm)

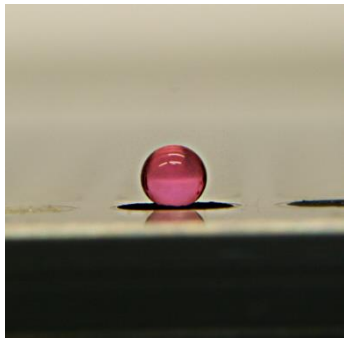
Powdered carbon aerogels



Carbon aerogel

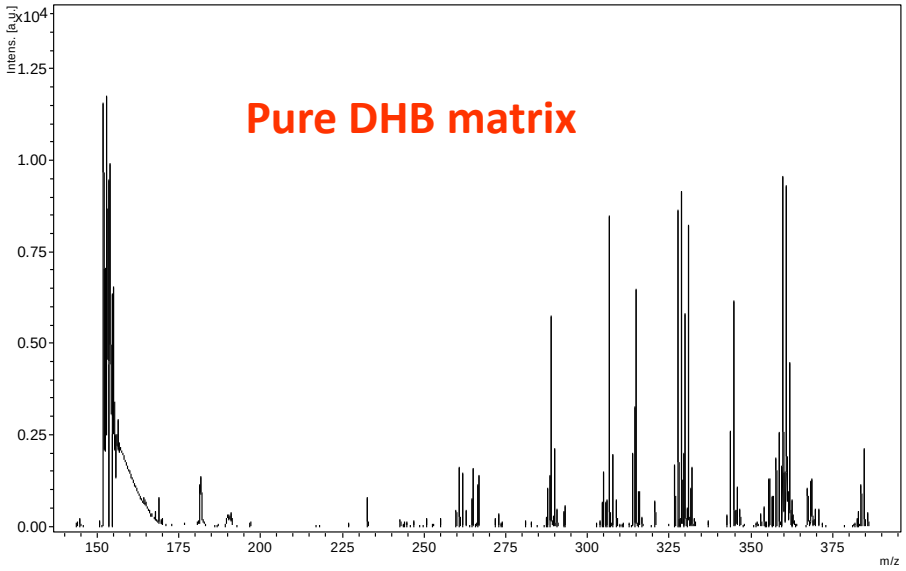


Powdering



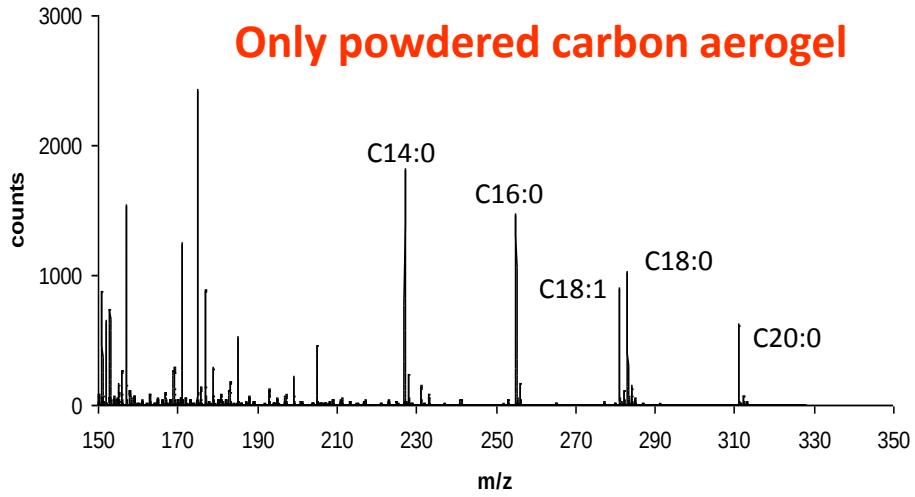
Powdered carbon aerogel

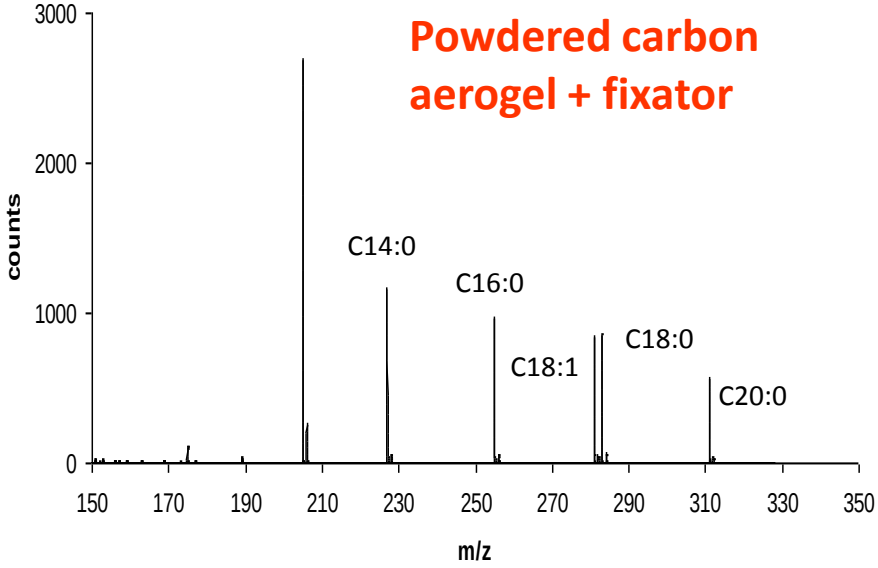
Fatty acids analysis



Negative mass-spectra of standard mixture of 5 free fatty acids obtained with different matrices.

All measurements were carried out at the same experimental conditions.





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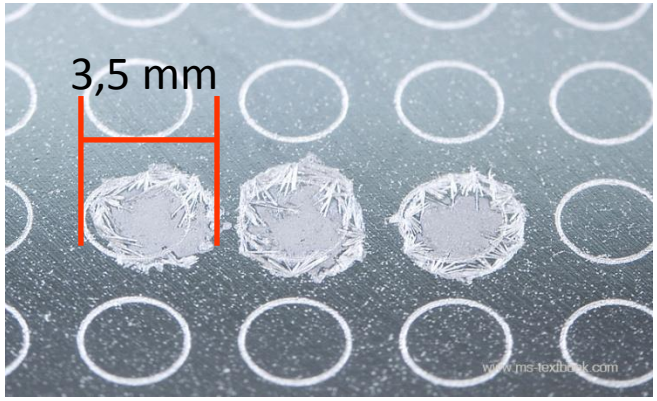
PROBLEM



Low reproducibility

SOLUTION

Microspots formation (≤ 1mm)

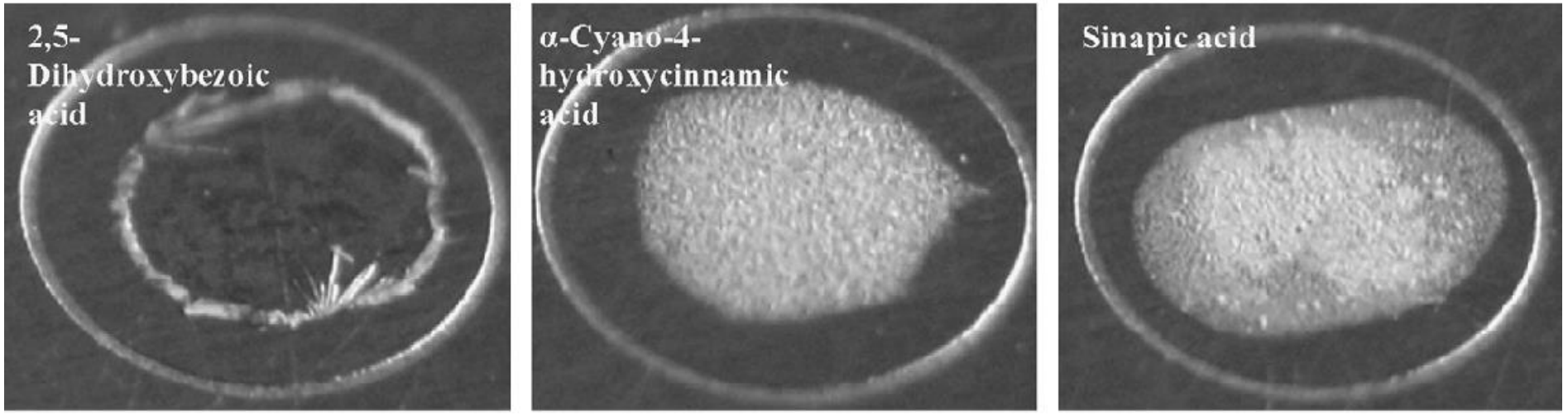
Commercial MALDI plates

Bruker Scout 384 TM anchor chip MALDI target

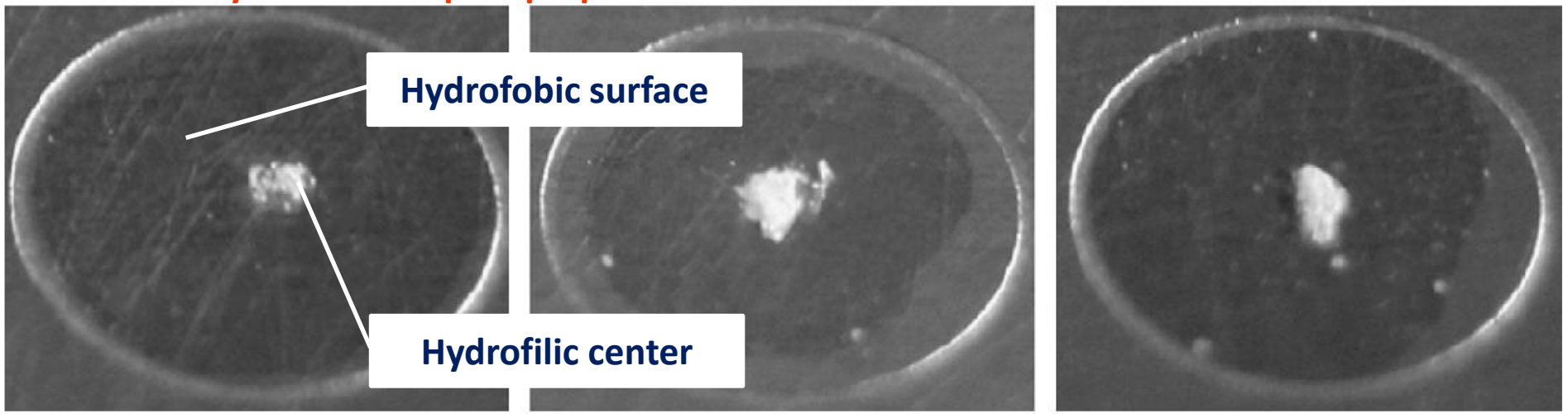
~1300 EUR

On-plate microspots formation

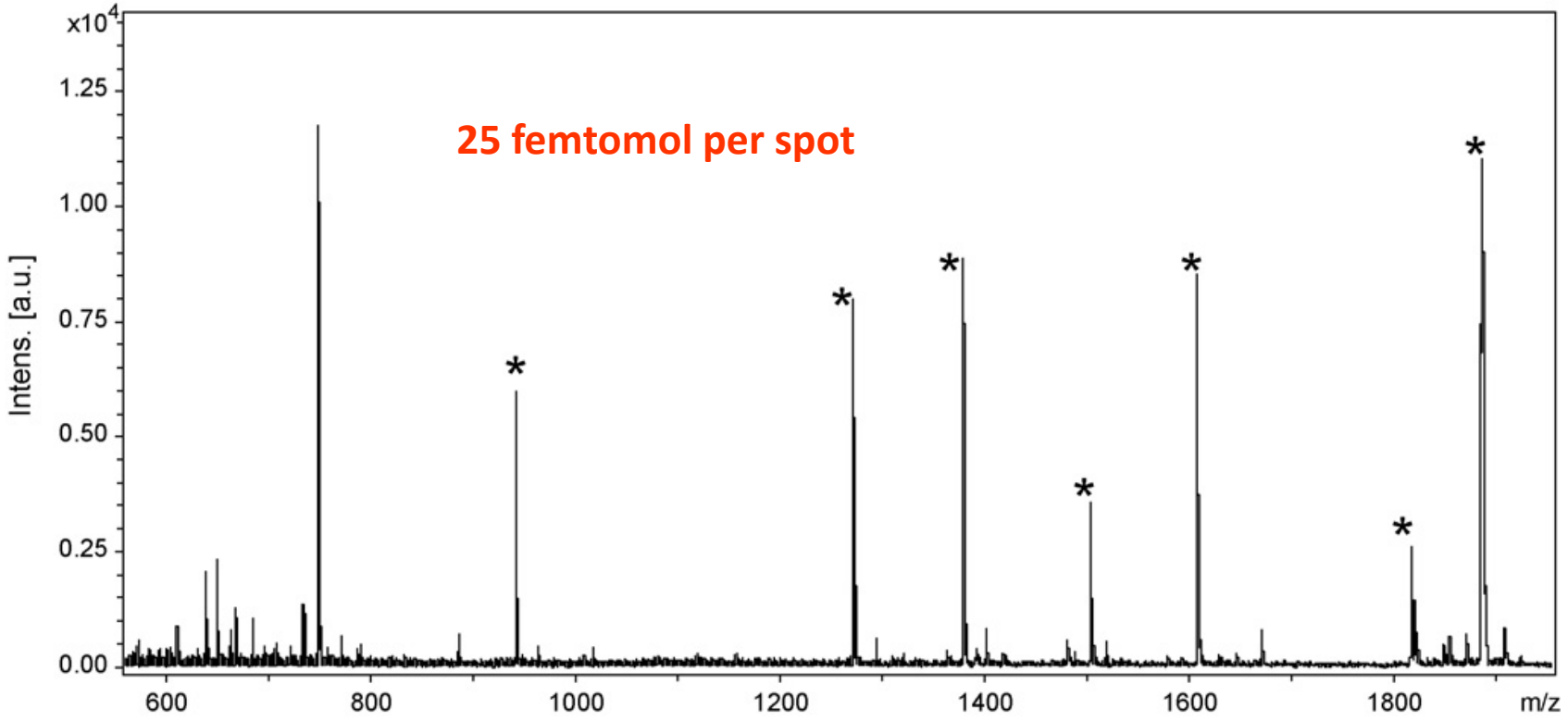
Conventional way of MALDI spots preparation



Modified way of MALDI spots preparation



MALDI mass spectra of a tryptic digest of apomyoglobin



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PROBLEM

MALDI MS hyphenation with CE system

SOLUTION

Off-line conection

Why hyphenation?

Capillary Electrophoresis - is the most efficient separation technique available for the analysis of both large and small molecules.

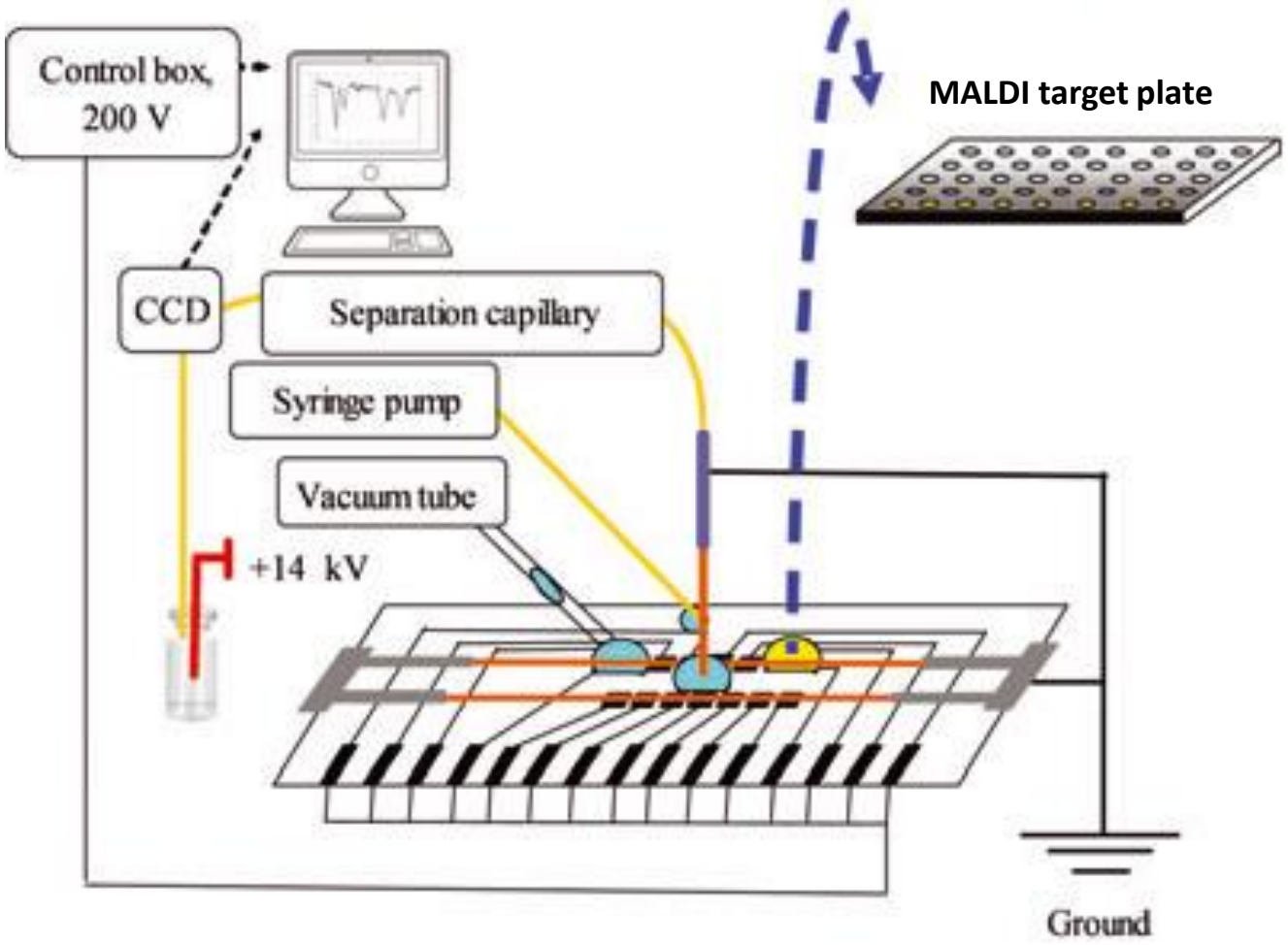
+

MALDI MS - is a key technique in mass spectrometry . Method is extremely sensitive, rapid, easy-to-operate, and relatively tolerant to contaminants.

=

CE -MALDI MS offers an opportunity to separate and identify analytes with potential for automation and high throughput, by using the benefits from both techniques.

Capillary electrophoretic system coupled with DMF board

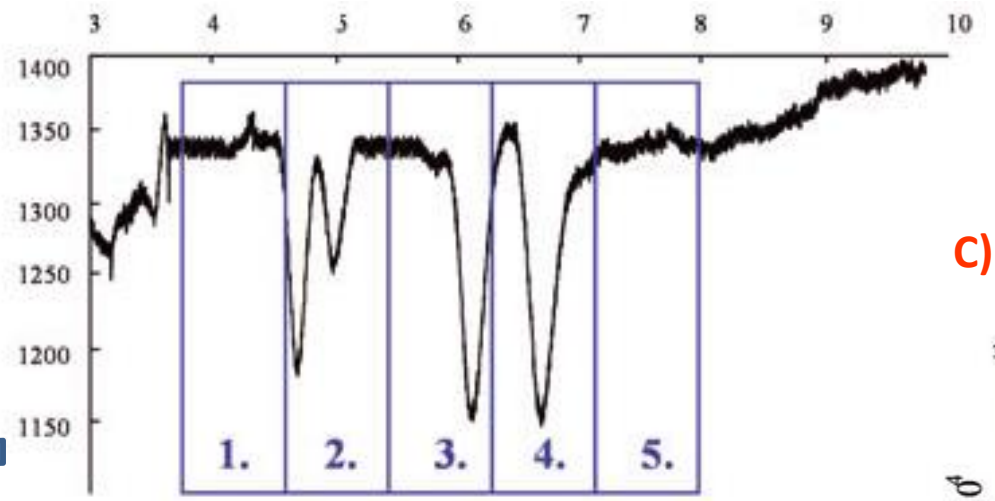


1. Separation of the analytes was achieved by capillary electrophoresis (CE)
2. Collected CE fractions were moved to predetermined position.
3. After fraction drying the MALDI analysis was carried out.

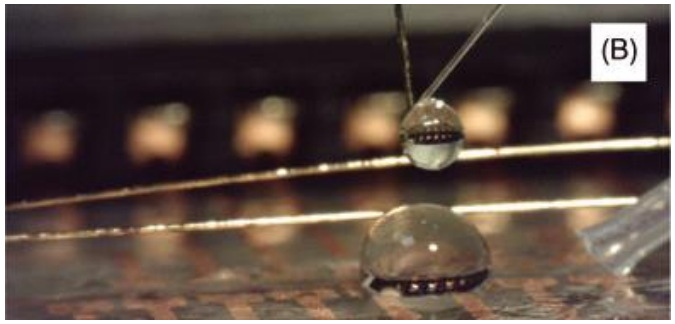
J. Gorbatsova, M. Borissova et al./*Electrophoresis* 2012, *33*, 2682–2688

Peptide identification by CE-MALDI MS

A) Separation by Capillary Electrophoresis

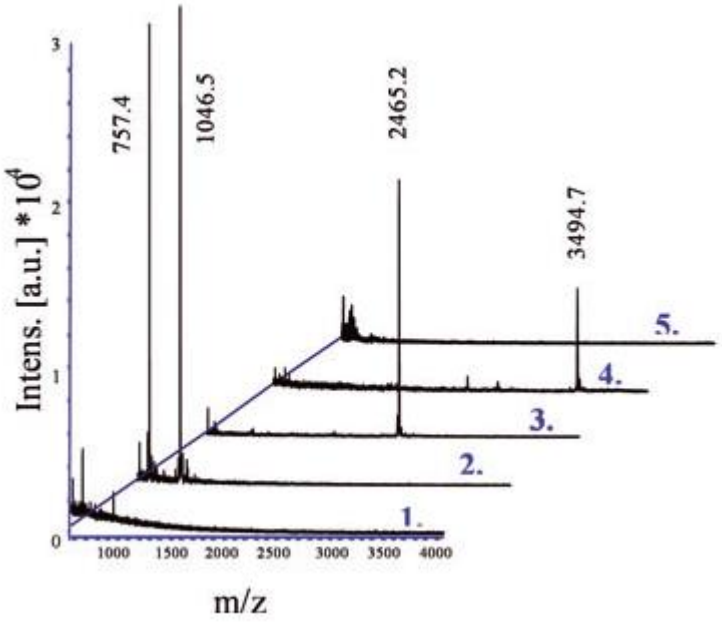


B) Fraction collection



3 µL droplets

C) Fraction analysis by MALDI



Summary

- ✓ extremely small amount of sample
- ✓ picomole-to-femtomole sensitivity due to sample concentration effect
- ✓ excellent signal reproducibility
- ✓ applicable for large and small molecules
- ✓ good choice for hyphenation systems

MALDI and biomass processing

Cyclic and non cyclic aliphatic–aromatic polyesters derived from biomass

Characterization of recalcitrant lignocellulosic biomass degradation

Production of oligosaccharides from extruded wheat and rye biomass using enzymatic treatment

Direct analysis of cellulose

…

Thank you very much for your attention!

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