

# Hmotnostní spektroskopie

Princip – separace iontů podle hmotnosti a náboje

- Fáze
- ionizace
  - urychlení iontů
  - analýza dráhy
  - detekce iontů
  - vyhodnocení

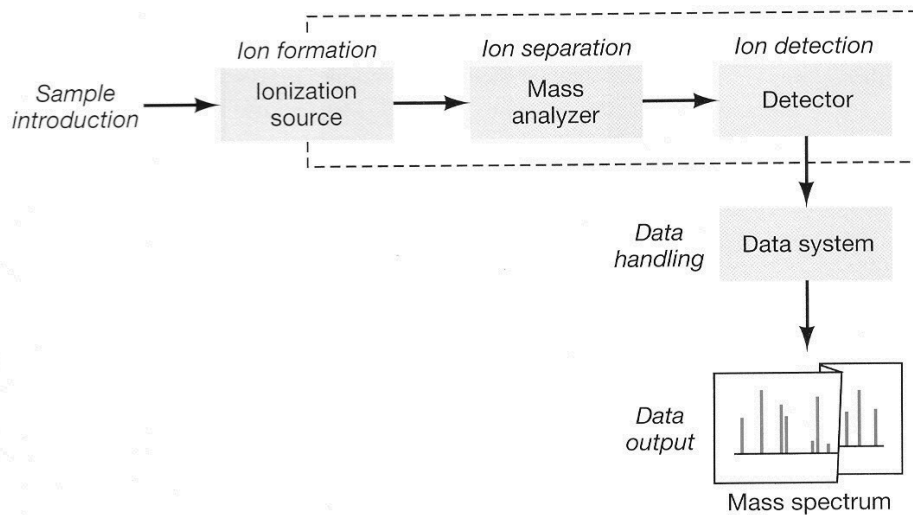
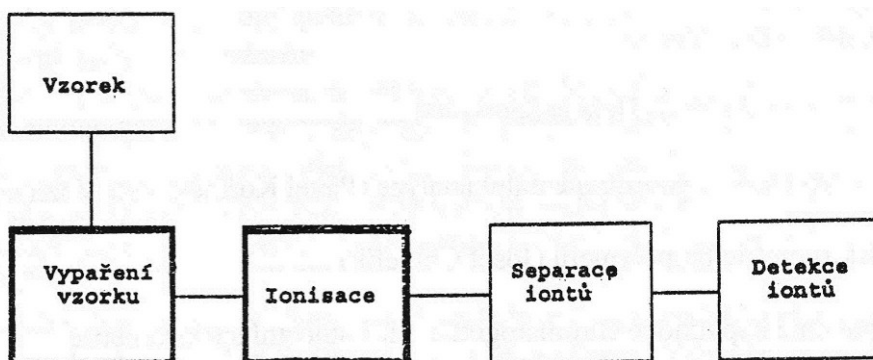
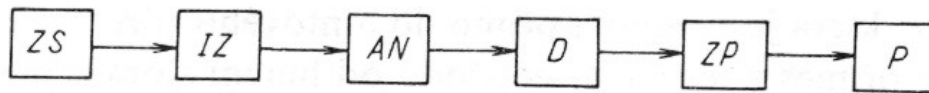


FIGURE 8.2. Modular components of a mass spectrometer. (Adapted, with permission, from [www.asms.org](http://www.asms.org) [Fig 1] the American Society for Mass Spectrometry [©1998 ASMS; What is Mass Spectrometry, 3rd edition].)





Obr. 3.110 Blokové schéma hmotnostního spektrometru

ZS — zaváděcí systém, IZ — iontový zdroj, AN — analyzátor, D — detektor,  
ZP — zapisovač, P — počítač

## Zavádění

Těkávké kapaliny – 2l nádoba, nástřík přes fritu či membránu, odpaření  
– molekulový otvor ca 10  $\mu\text{m}$

Málo těkávké v kapiláře do iontového zdroje

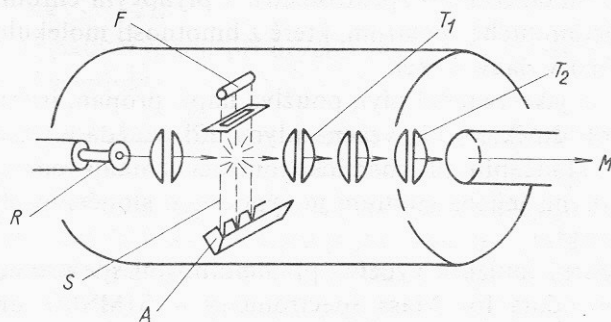
Spray (ESI)

Biopolymery – matrice

Spojení s CG i LC – separátory – oddělení mobilní fáze

## Ionisace

EI – ionizace dopadem elektronů



Obr. 3.112 Iontový zdroj pro ionizaci nárazem elektronů (Consolidated Electrodynamic Corp.)

F — žhavené vlákno, A — anoda, S — elektronový svazek, R — molekulární otvor,  
 $T_1$  a  $T_2$  — akcelerační štěrby, M — přívod do analyzátoru

ESI – ionizace elektrosprejem

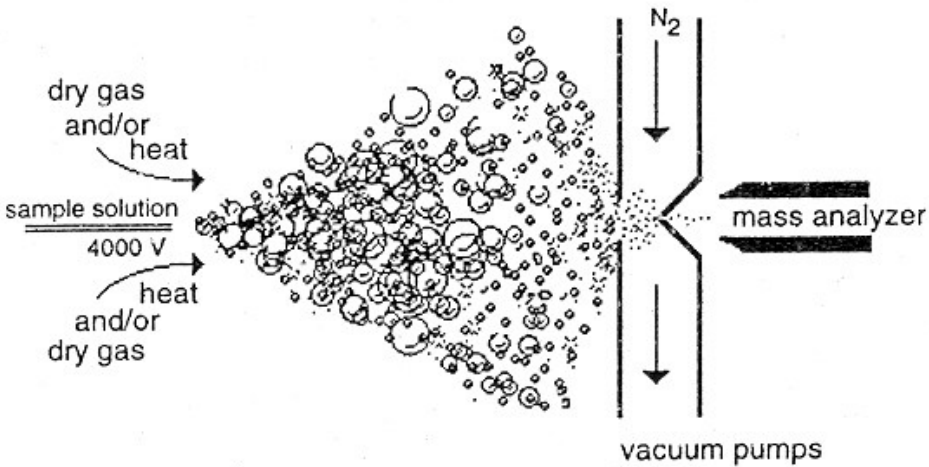
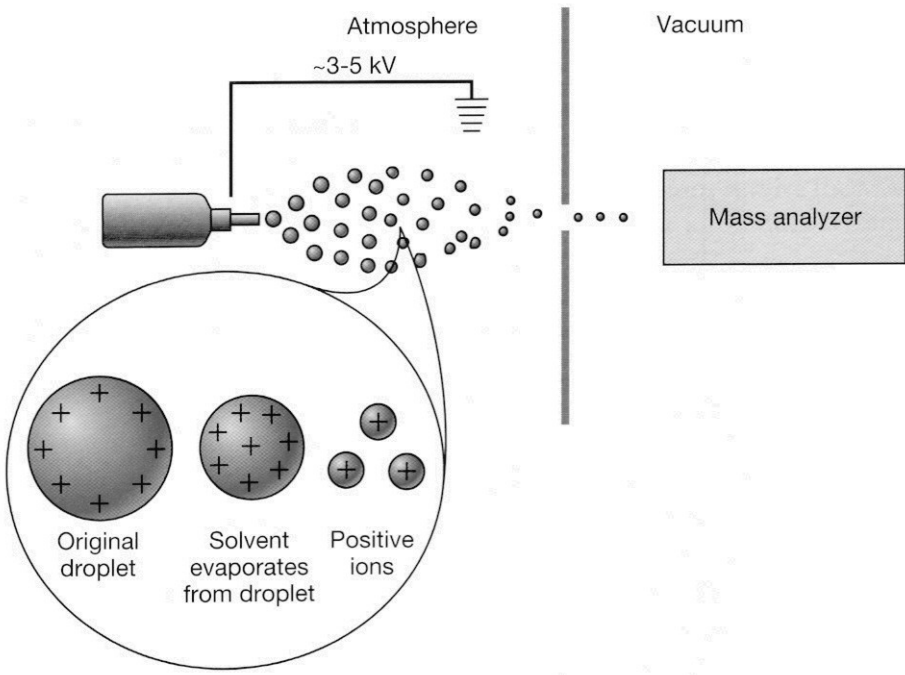


FIG. 1. ESI source.



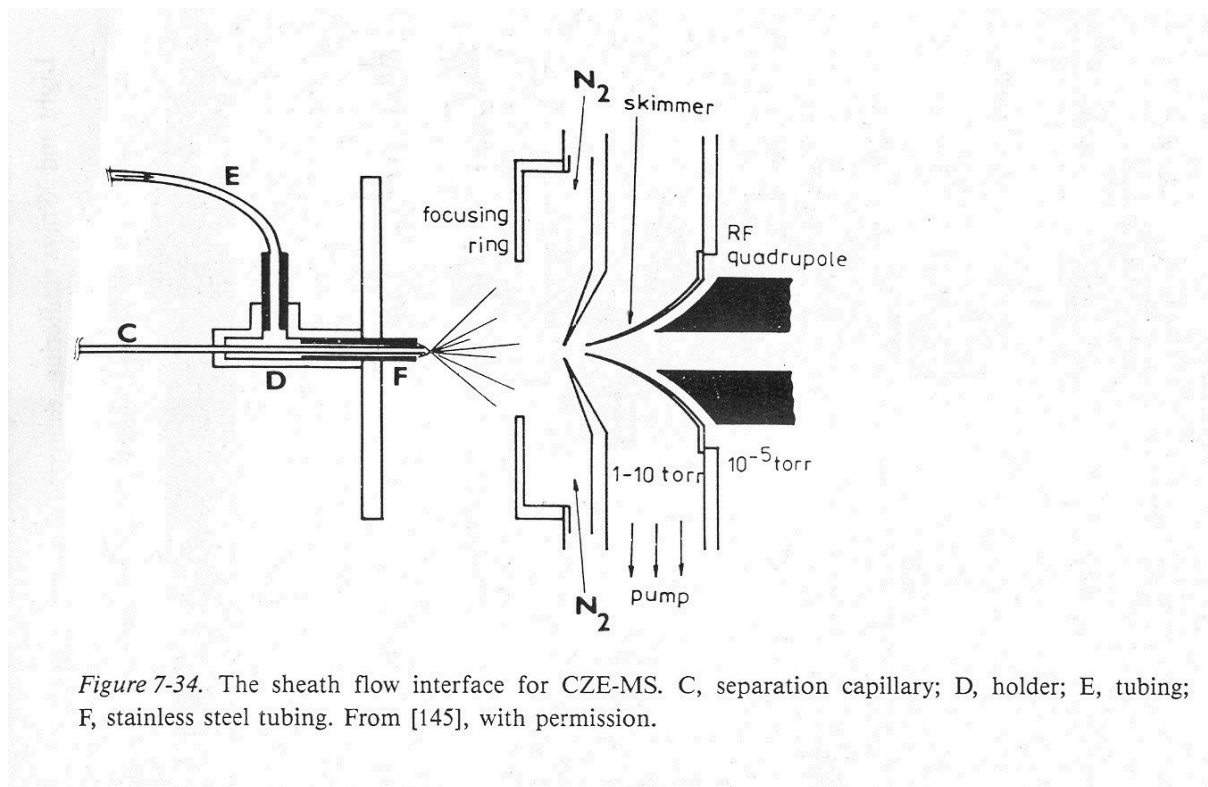


Figure 7-34. The sheath flow interface for CZE-MS. C, separation capillary; D, holder; E, tubing; F, stainless steel tubing. From [145], with permission.

## MALDI

(matrice - kys. 2,5-dihydroxybenzoová, 5-chlorsalicylová,  $\alpha$ -kyano-4-hydroxykořicová, nikotinová, apod.)

-směs na Ag plíšku

mechanismus – adice či eliminace  $H^+$  - možnosti 2 módů –  $A^-$  nebo  $B^+$

Hmotnostní limity ESI-MS: 200 000 (prakt 70000), MALDI-MS > 300 000 (150 000)

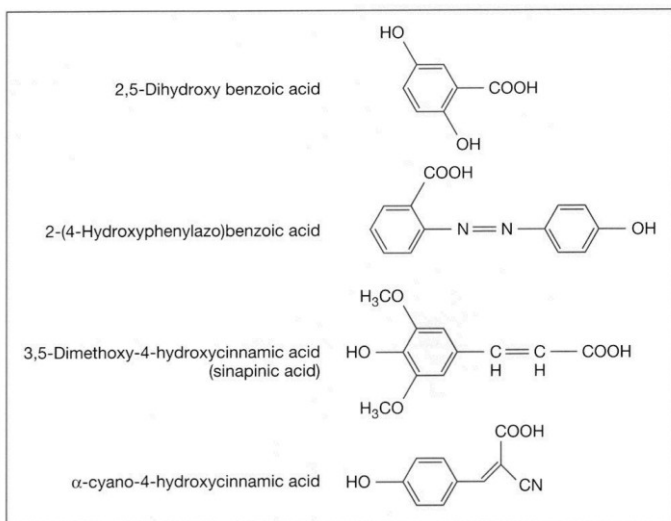
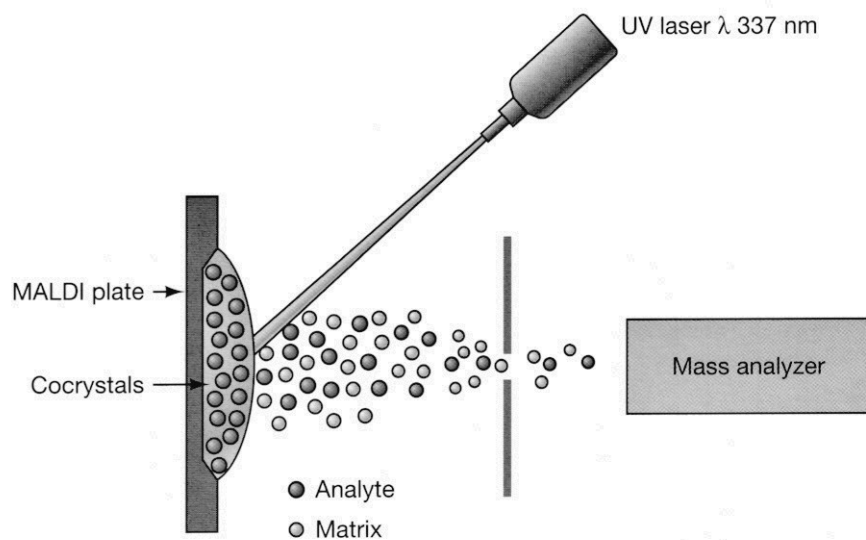


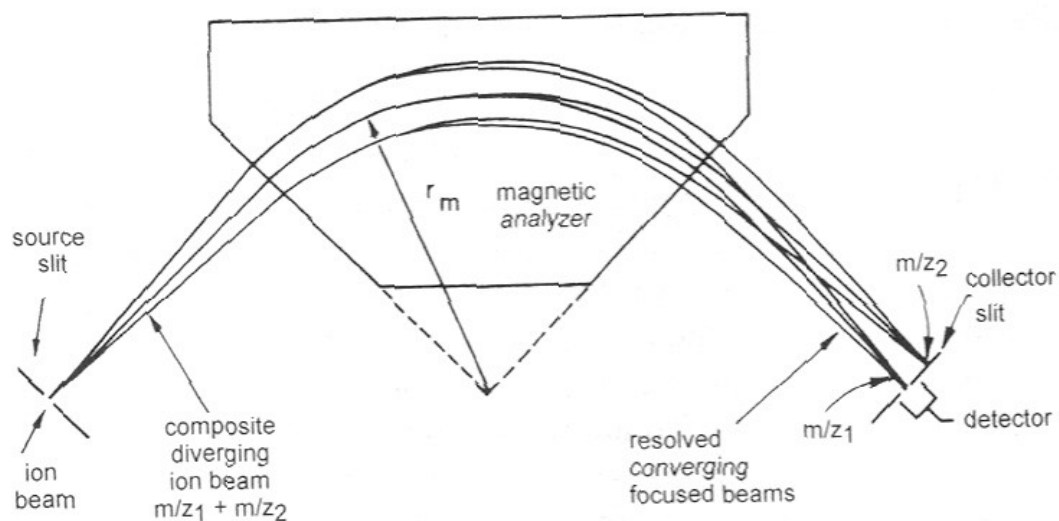
FIGURE 8.7. Chemical structures of some commonly used matrices.

## Separace iontů

Urychlení - elektrody

## Rozdělení prostorové nebo časové – analýza dráhy

### Zakřivení dráhy v elektrickém a magnetickém poli



**Fig. 4.19.** A 90° magnetic sector illustrating  $m/z$  separation and direction focusing in a plane (angles are shown exaggerated). Reprinted from Ref. [1] with permission. © American Association for the Advancement of Science, 1979.

$$E = mv^2/2 = q \cdot U$$

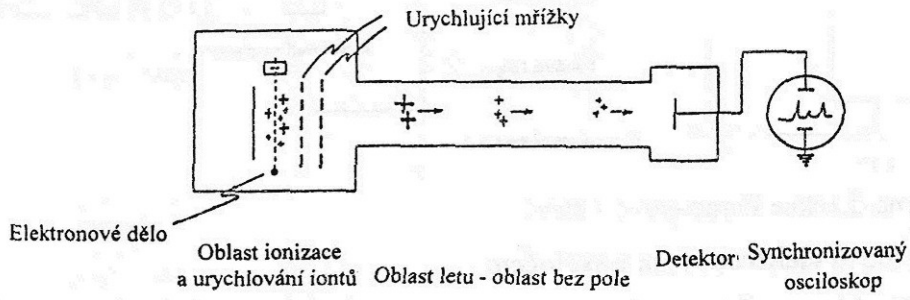
$$F = mv^2/r = B \cdot q \cdot v$$

$$m/q = r^2 \cdot B^2 / 2U$$

### Přeletový – měření času letu (TOF – time of fly)

$$E = mv^2/2 = q \cdot U$$

$$m/q = 2U/v^2 = 2Ut^2/l^2$$



OBR.10. Schema hmotnostního spektrometru TOF

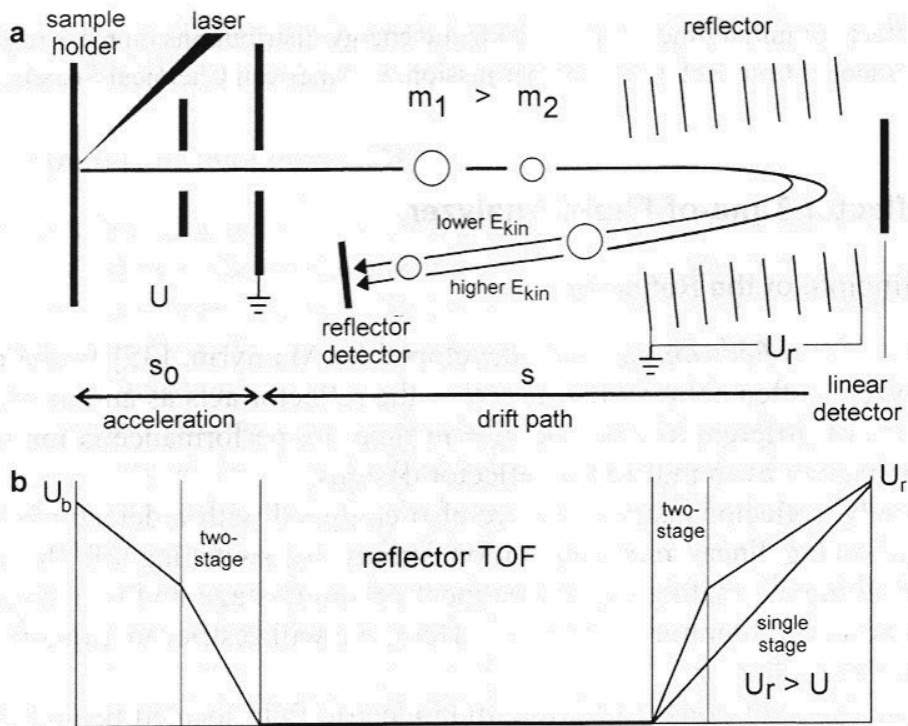
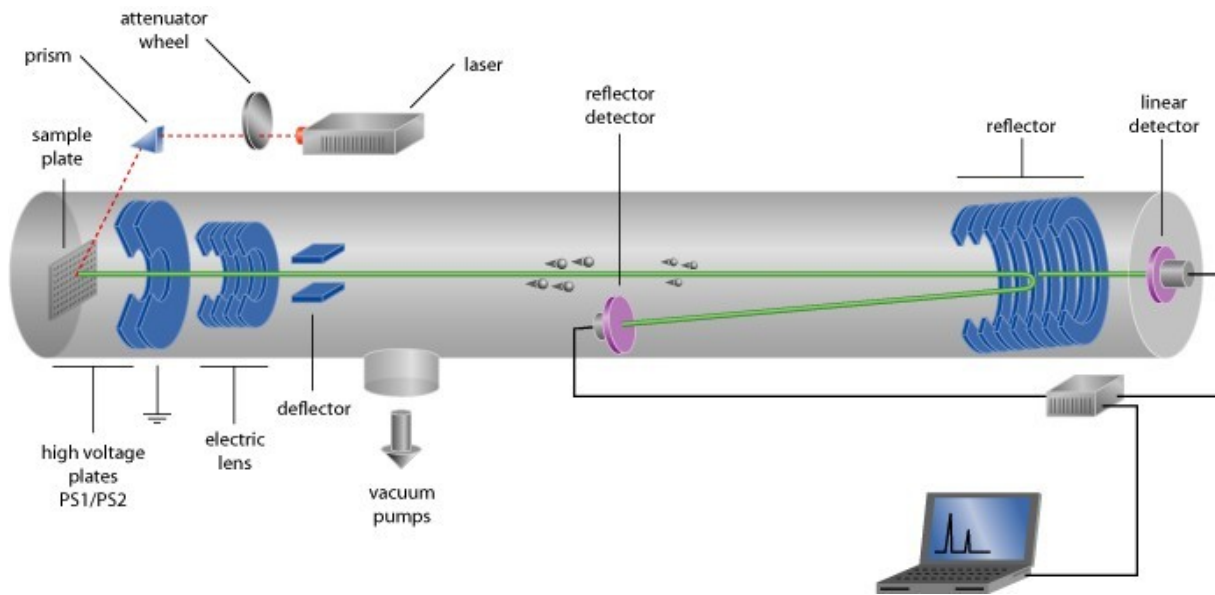
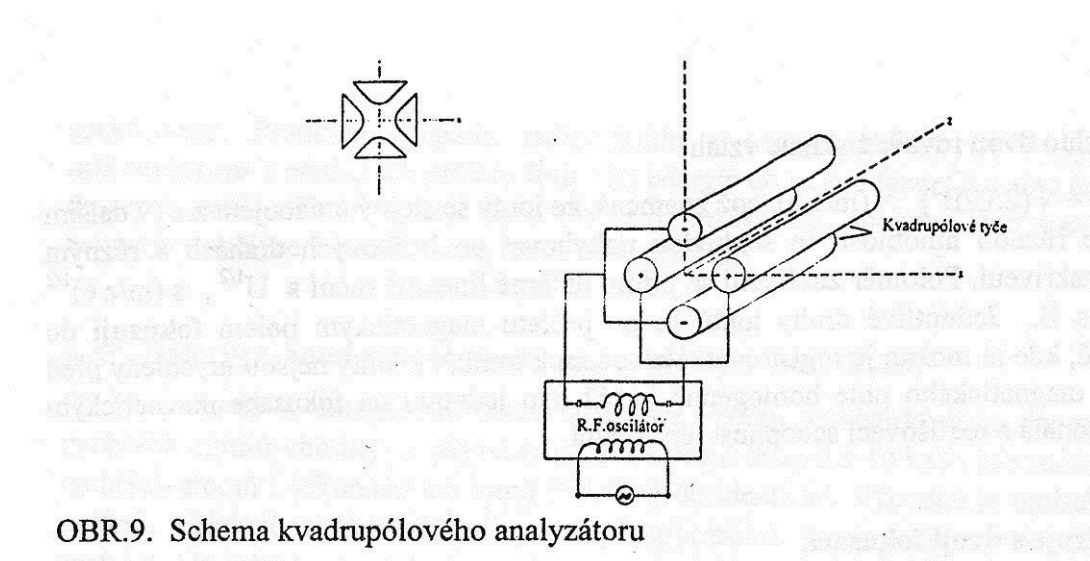


Fig. 4.6. Schematic of a ReTOF (a) and potentials along the instrument (b).



Radiofrekvenční – kvadrupólový, iontové pasti

$$m/q = 266 \cdot 10^9 \cdot U / f^2$$



OBR.9. Schema kvadrupólového analyzátoru



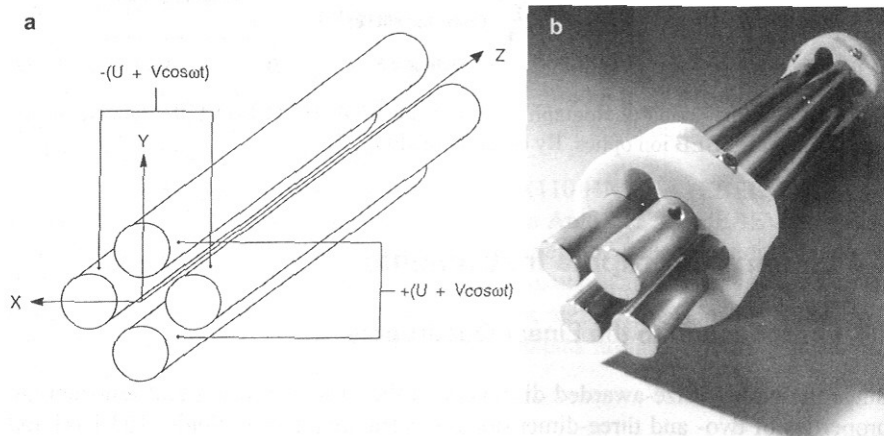


Fig. 4.32. Schematic (a) and photograph (b) of a linear quadrupole mass analyzer. By courtesy of JEOL, Tokyo (a) and Waters Corp., MS Technologies, Manchester, UK. (b).

[137]

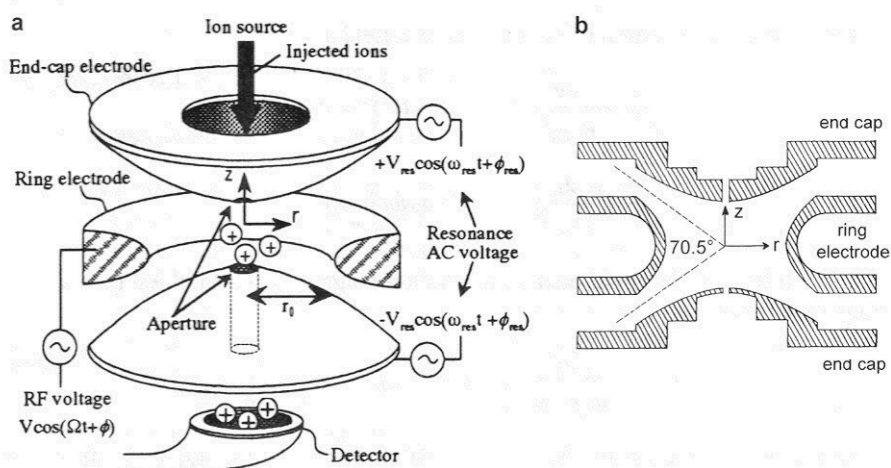
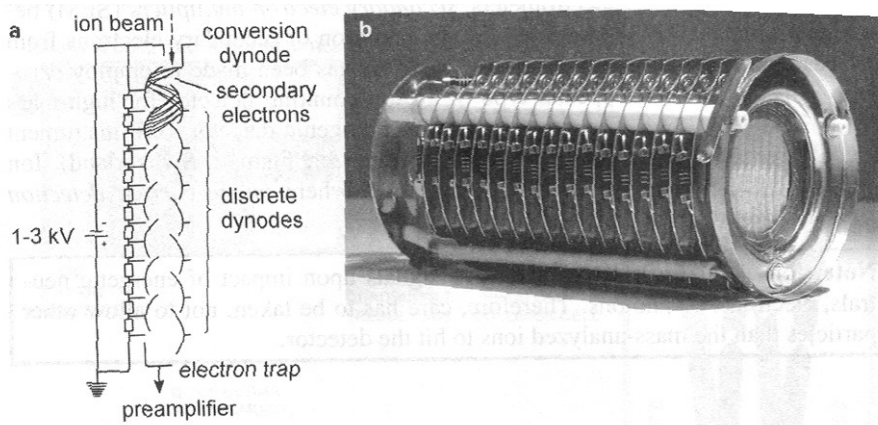
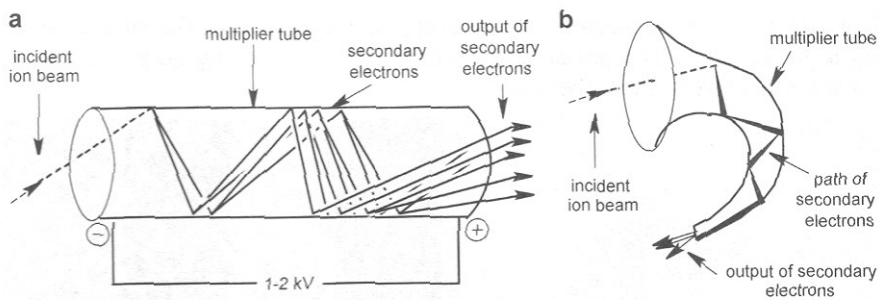


Fig. 4.40. Schematic of a quadrupole ion trap. (a) QIT with external ion source (illustration stretched in  $z$ -direction) and (b) section in the  $rz$ -plane (in scale). (a) Reproduced from Ref. [144] by permission. © John Wiley & Sons, 2000.

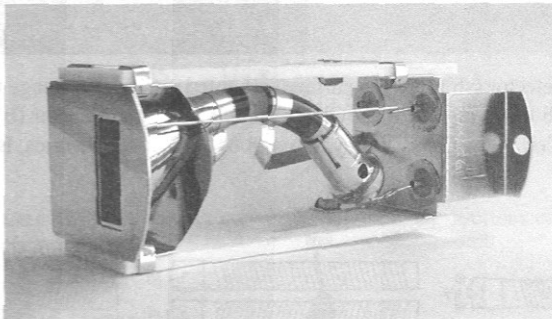
# Detektory



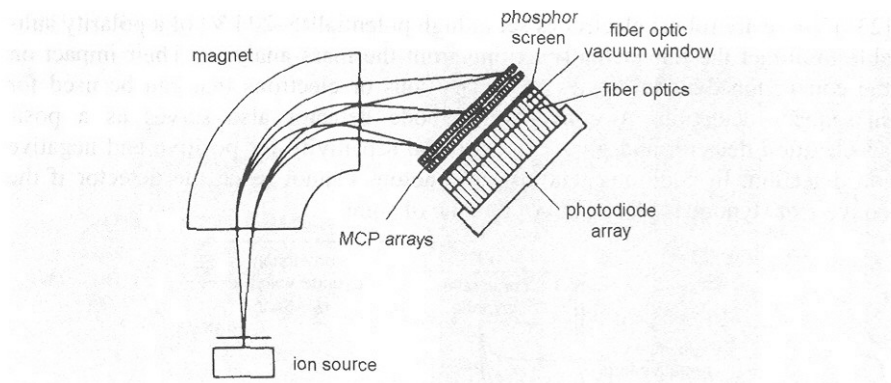
**Fig. 4.57.** Discrete dynode electron multipliers. (a) Schematic of a 14-stage SEM. (b) Photograph of an old-fashioned 16-stage Venetian blind-type SEM clearly showing the resistors and ceramics insulators between the stacking dynodes at its side. (a) Adapted from Ref. [238] by permission. © Springer-Verlag Heidelberg, 1991.



**Fig. 4.58.** Schematic of linear channel electron multiplier (a) and curved channel electron multiplier (b). By courtesy of Waters Corporation, MS Technologies, Manchester, UK..



**Fig. 4.59.** Photograph of a channeltron multiplier.



**Fig. 4.63.** Array detector in the focal plane of a magnetic sector to detect a small mass range simultaneously. By courtesy of Thermo Electron (Bremen) GmbH.

## Využití

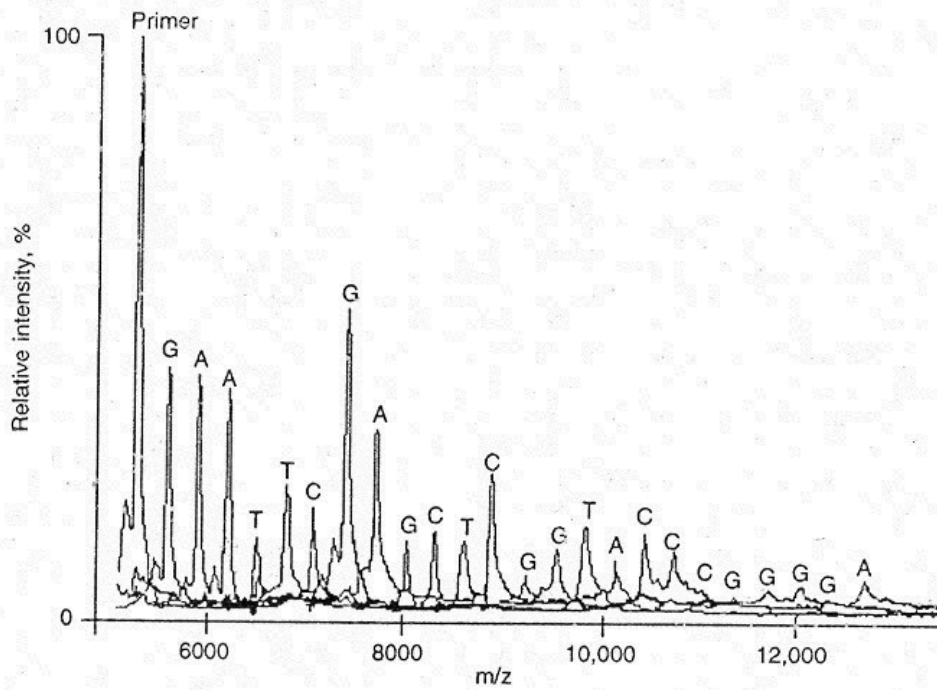
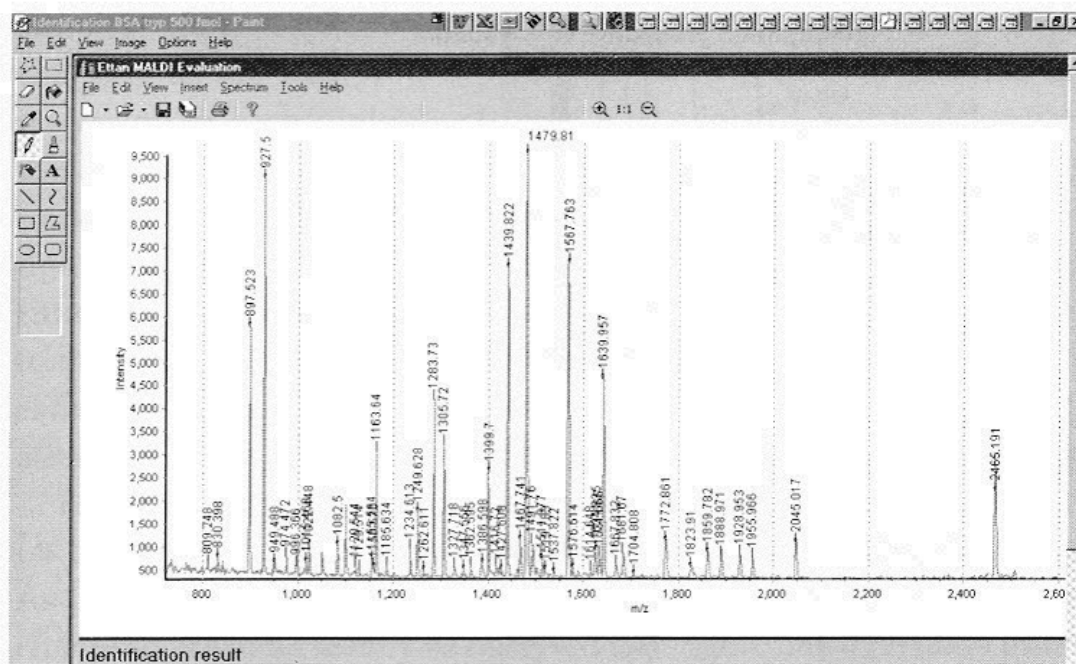
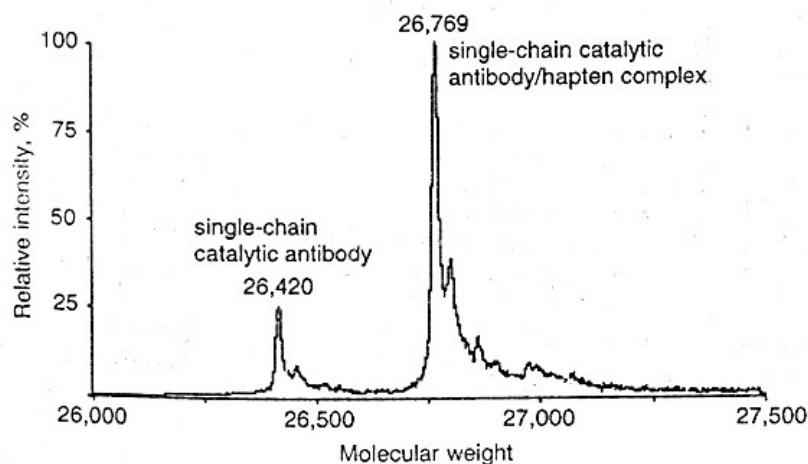


FIG. 12. Negative-ion MALDI mass spectra of synthetic oligonucleotides corresponding to mock A, C, G, and T sequencing reactions. The order of the peaks corresponds to the sequence. Reproduced with permission from ref. 64 (copyright Wiley, Sussex, U.K.).



**Fig. 53:** MALDI in positive ion mode for the analysis of a tryptic digest of myoglobin. The spectrum was acquired using  $\alpha$ -cyano-4-hydroxycinnamic acid as the matrix. Each of the peptide

signals observed in the mass range  $m/z$  800–2500 exist as the  $[M+H]^+$  molecular ion, where  $M$  is the mass of the peptide. A MALDI-ToF spectrum of a BSA tryptic digest.



**FIG. 8.** Noncovalent single-chain catalytic antibody-hapten complex as observed with chemically assisted electrospray (ion spray) mass analysis. Reproduced with permission from ref. 34 (copyright American Chemical Society, Washington, DC).

# Kombinace

Online detekce

Tandemy

