HPLC and CE detectors

UV (no specificity)

Z shaped detector

Fluorescence (no specificity, but more sensitive)

Electrochemical (can be very sensitive, but no specificity)
Voltammetry, coulommetry

Mass Spectrometry – electrospray (sensitive, provides MW and structural info)

Effluent from the HPLC column is directed into a metal capillary. A ≈ 5 kV is applied to the capillary. As a result, the solution is sprayed out of the capillary. In the process H^+ ions are formed through an electrochemical reaction at the capillary surface. The solvent undergoes rapid expansion and a process of evaporation. As the droplets of water evaporate, their charge density increases. At some point the charge density becomes unstable and the droplet undergoes a coulombic explosion forming many smaller droplets. This process is repeated until all of the solvent evaporates. Finally what is left are intact (multi-) protonated gas-phase solute ions. These ions are focused into the mass analyzer, where their mass-to-charge ratios are measured

Fig 25-30

Soft-ionization technique

MS-MS experiment to obtain fragments that can provide structural info.

Isolate the ion of interest in the mass analyzer, excite it, cause it to fall apart, and measure the mass-to-charge ratio of the fragments

Handout

CE

Set-up
Separates charged species

Retention time of the solute is determined by its apparent mobility.
Apparent mobility (μapp) is the sum of the electrophoretic mobility (μep) and the electroosmotic flow rate (μeo).

$$\mu app = \mu ep + \mu eo$$

electrophoretic mobility – The μep of a solute depends upon its charge and size.
Negatively charged molecules flow toward the positively charged electrode, and this flow is assigned a negative sign. Positively charged molecules flow toward the negatively charged electrode, and this flow is assigned a positive sign.
The absolute magnitude of the electrophoretic mobility increases with increasing charge and decreasing size.

Electroosmotic flow – This is the bulk flow that results in a capillary electrophoresis experiment from a build up of positively charged ions at the surface walls of the capillary. The build-up occurs as positively charged ions are attracted to negatively charged groups fixed at the walls of the capillary, such as SiO_2^-. This concentration of positive charges at the surface, create a mass movement towards the negatively charged electrode (cathode). This mass movement drags the whole solution with it. Thus, even neutral species are flow at the electrosmotic (bulk) flow.
On bare silica, the electroosmotic flow always carries a positive sign.
Plug-like flow (Fig 26-16)

Sample introduction:
Hydrodynamic - small pressure pulse
Electrokinetic – use high voltage (μap) to introduce sample

Inherent problem with CE is concentration sensitivity
* Small capillary
– 20 cm of a 50 μm ID column
- internal volume = 20 cm (3.14)(0.0025 cm)^2 = 390 nl

For good separation the width of the sample plug should be no more than 2 % of the column volume or 8 nl.

- Modern mass spectrometers can detect 20-200 fmol of a solute. 20 fmol in 8 nl is a concentration of 2.5 μM, a fairly high concentration.

- Stacking and other attempts to pre-concentrate the sample
  Solutes migrates faster in a solution of low conductivity

Off-line
Filters (magnet) that hold C18 stationary phase in place

Types of capillary electrophoresis

Capillary zone electrophoresis (CZE)

The experiment described above

Capillary isoelectric focusing

Uses amino acid like amphiprotic species of a wide range of PIs are used to set up a pH gradient, and solutes are separated on the basis of their PIs.

Micellar electrokinetic capillary chromatography

Micelles added to the buffer – sodium dodecyl sulfate (fig 26-26)
Neutrals can be separated.

Capillary electrochromatography

Uses an HPLC like packing in conjunction with CZE.
Can be problematic – bubble formation
Neutrals can be separated.

Capillary gel electrophoresis

Separates by size. Diffusion in and out of a porous gel. Better resolution than size-exchange chromatography for some applications (DNA pieces).