Separation and analysis of biological probes: methods and instruments

Capillary electrophoresis

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Capillary electrophoresis

Capillary electrophoresis is a family of electrokinetic separation methods performed in submillimeter diameter capillaries or in micro- and nanofluidic channels. Capillary electrophoresis can be assigned to a group of capillary zone electrophoresis techniques.

In capillary electrophoresis approaches, analytes migrate through electrolyte solutions under the influence of an electric field.

Analytes can be separated according to ionic mobility and/or different ability to interact with a stationary phase via non-covalent interactions; may be concentrated ("focused“) by means of gradients in conductivity and pH.

Advantages:

High-speed analysis; Very small sample size (2-30 nL from 3-5 μl);
High separation ability; High sensitivity
Quantitative analysis Automation
Low consumption of reagents (<1% of HPLC)

The development of capillary electrophoresis is often attributed to **James W. Jorgensen and Krynn DeArman Lukacs**, who first demonstrated its capabilities.

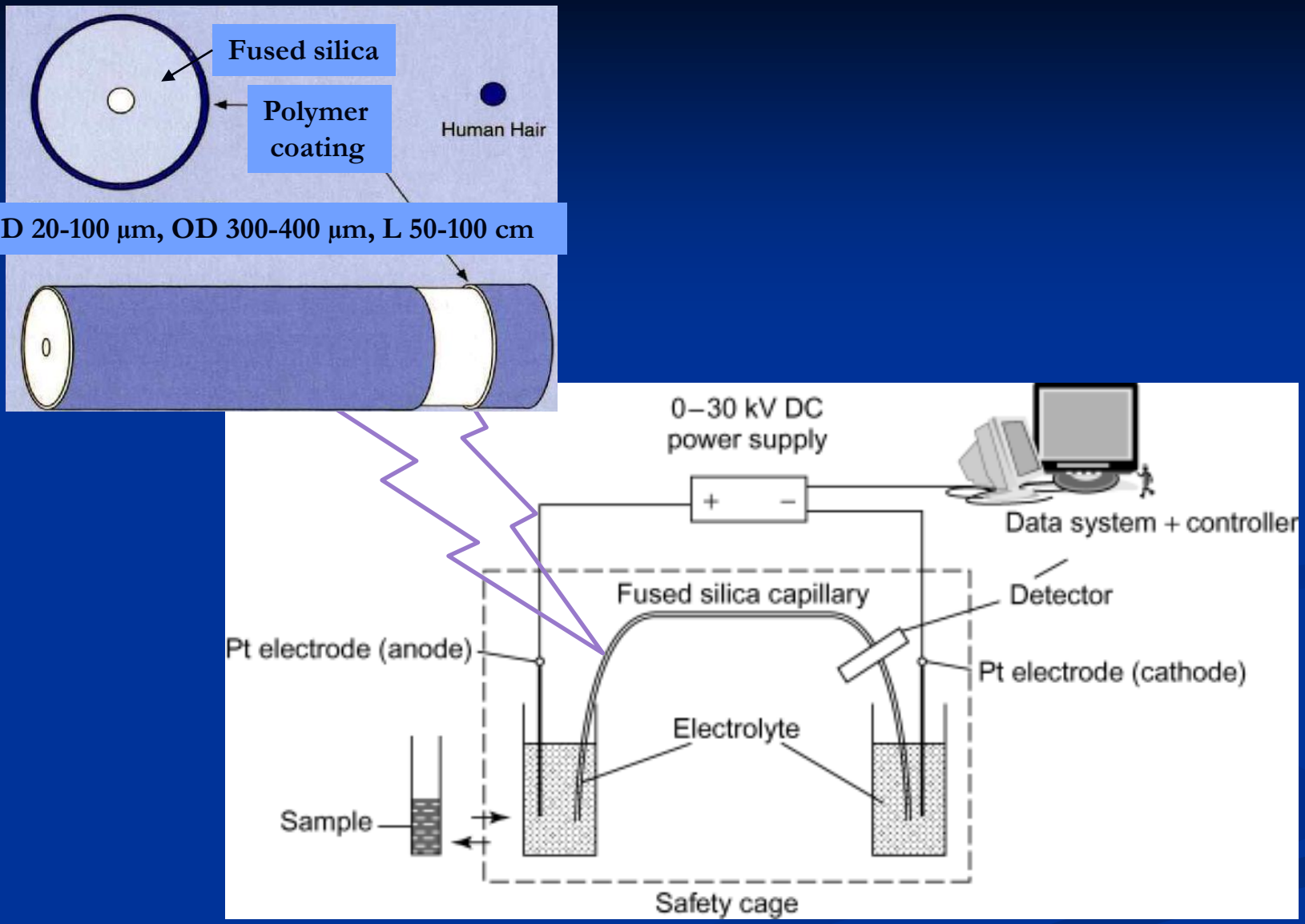
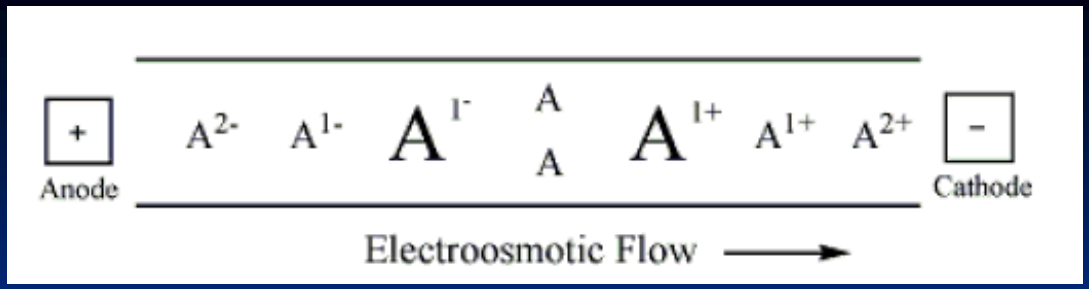


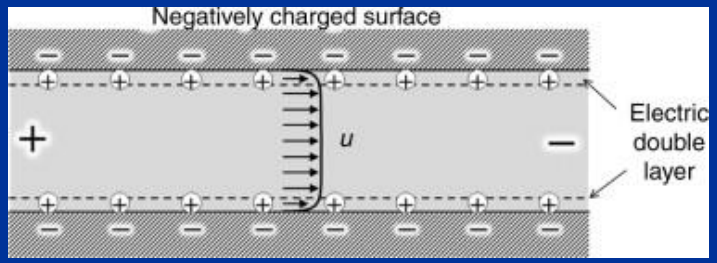
Diagram of capillary electrophoresis system



The electrophoretic migration velocity (M_e) of a particle in an electric field is described by

$$v_e = qE/(6\pi r \eta),$$

where q – net charge of analyte, E – electric field strength, r – Stokes radius, η –viscosity of the solvent



electro-osmotic flow

$$v_{EOF} = \varepsilon \zeta_0 E/ \mu$$

v_{EOF} electro-osmotic velocity, ε- relative permittivity of the fluid, ζ_0 (V) - zeta potential at the wall, E - electric field, μ - dynamic viscosity of the fluid.

if V=300 V (1-cm microchannel, r = 50 μm), $\varepsilon = 7.1\times10^{-10}$ F/m, ζ_0 = -0.1 V, μ=0.001 N s/m^2

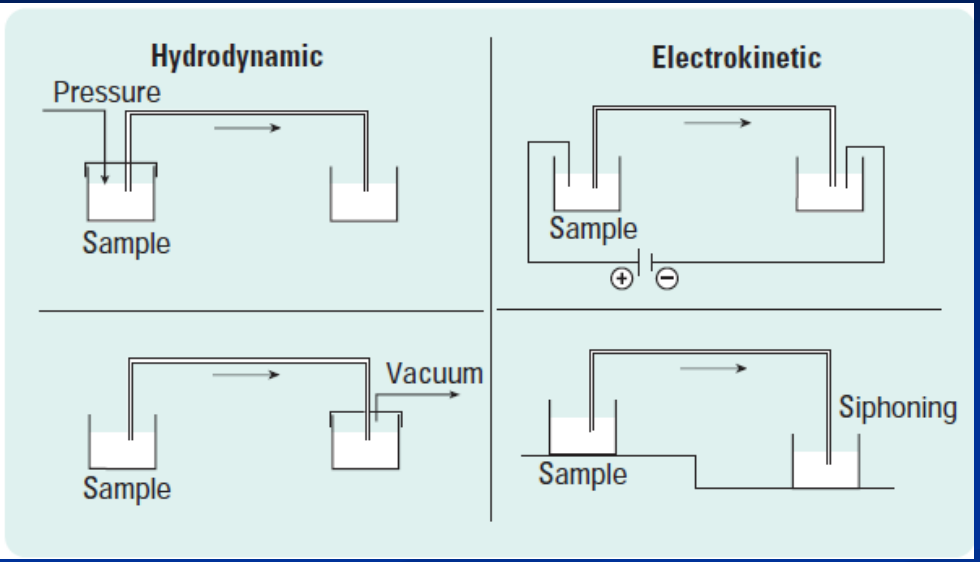
v_{EOF} = 2.13 mm/s; volumetric flow rate Q = 1 μL/min

- High electrical field (100 to 500 V/cm) can be applied due to high electrical resistance of the capillary
- Generated heat dissipates efficiently due to large surface area-to-volume ratio
- High electrical field results in short analysis times and high resolution

Capillary electrophoresis

Loading of analyte into the capillary via :

- capillary action,
- pressure,
- siphoning,
- electrokinetically (with low voltage)



Types of detection:

- UV or UV-Vis absorbance,
- Fluorescence (sensitivity 10^{-18} – 10^{-21} mol; multicolor detection)
- SERS-based detection (SERS-active substrates for vibrational spectroscopy analysis are used)
- Mass spectrometry (electrospray ionization at the capillary outlet and further detection with mass spectrometer)

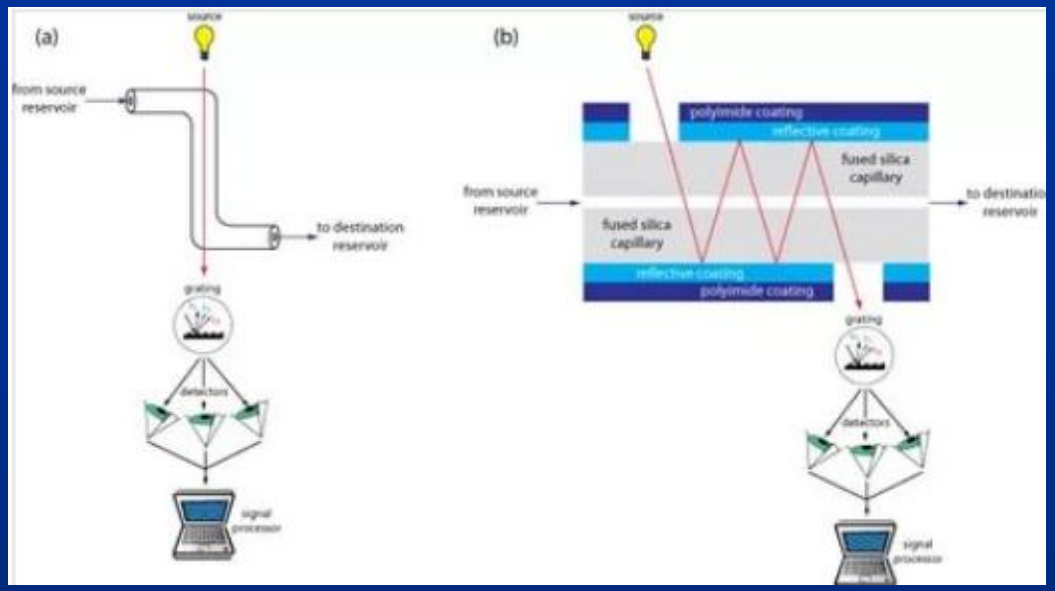
Capillary electrophoresis

Types of detection:

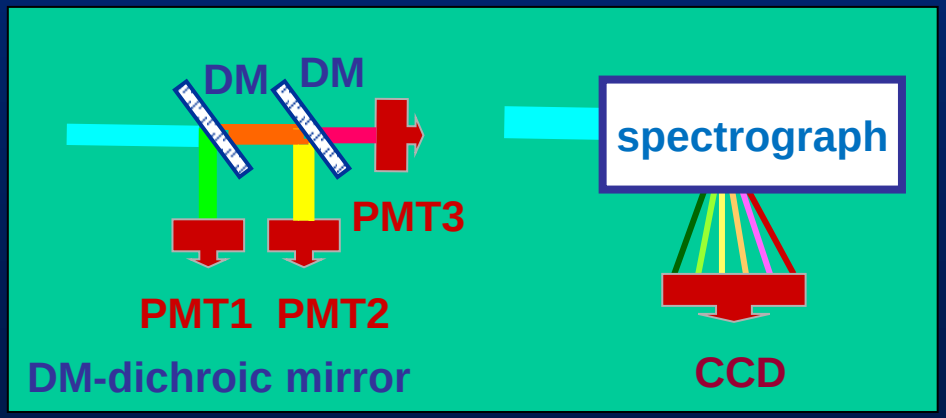
Method	Mass detection limit (moles)	Concentration detection limit (molar)*	Advantages/ disadvantages
UV-vis absorption	$10^{-12} – 10^{-15}$	$10^{-5} – 10^{-7}$	"Universal". DAD offers spectral information.
Fluorescence	$10^{-15} – 10^{-17}$	$10^{-7} – 10^{-9}$	Sensitive. Usually requires sample derivatization.
Laser induced fluorescence	$10^{-18} – 10^{-20}$	$10^{-9} – 10^{-12}$	Extremely sensitive. Usually requires sample derivatization. Expensive.
Amperometry	$10^{-18} – 10^{-19}$	$10^{-10} – 10^{-11}$	Sensitive. Selective but useful only for electroactive analytes. Not robust.
Conductivity	$10^{-15} – 10^{-16}$	$10^{-6} – 10^{-7}$	Universal.
Mass spectrometry	$10^{-16} – 10^{-17}$	$10^{-8} – 10^{-9}$	Sensitive. Structural information. Interface between CE and MS complicated.

- Mass spectrometry - electrospray ionization at the capillary outlet and further detection with a mass spectrometer

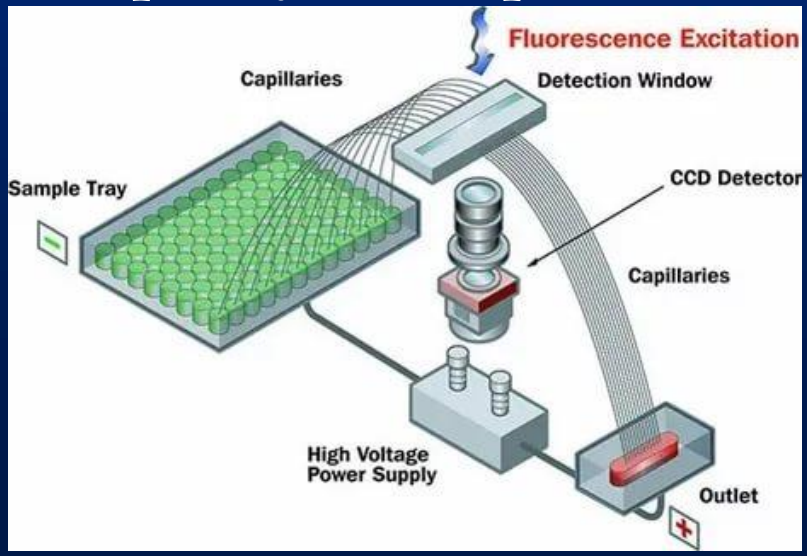
Enhancing sensitivity of UV-VIS absorption detection

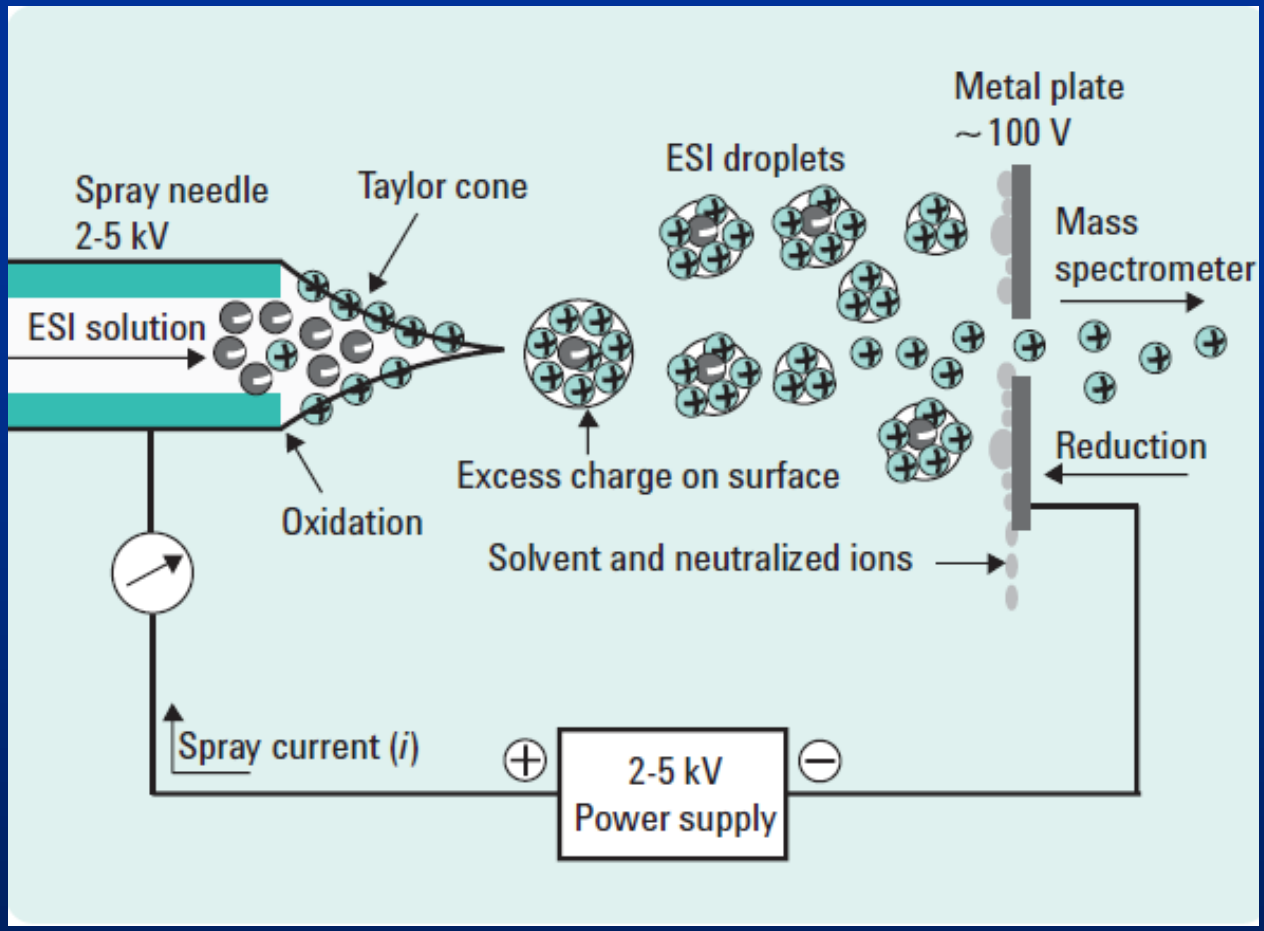


Multicolor detection of fluorescence



Multichannel capillary electrophoresis





Pictorial description of the electrospray ionization process.

Capillary electrophoresis techniques

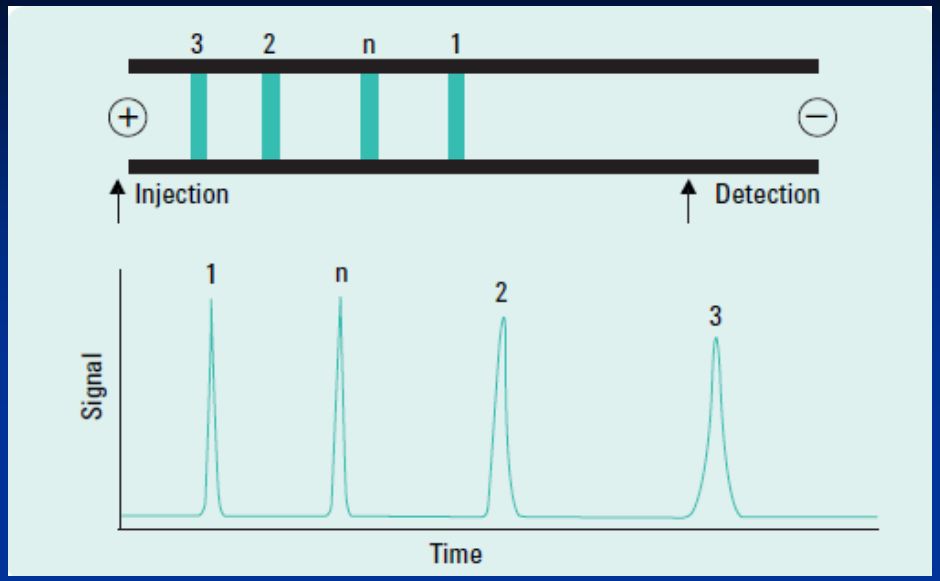
mode	principle of separation	analyte
capillary zone electrophoresis	charge-to-mass ratio	small ions, small molecules, peptides, proteins, DNA (limited)
capillary isoelectric focusing	isoelectric point; separation by movement through a stationary pH gradient in the run buffer	peptides, proteins
capillary isotachophoresis	Mobility: separates analytes with same mobility as the buffer ions, which differ at inlet and outlet	Small molecules peptides, proteins
micellar electrokinetic capillary chromatography	*Charged species: charge-to-mass ratio and partitioning into micelles according to hydrophobicity *Neutral species: partitioning into detergent micelles according to hydrophobicity	Small molecules peptides, DNA

Capillary electrophoresis techniques

mode	principle of separation	analyte
capillary gel electrophoresis **nondenaturating** **denaturating (SDS, urea)**	**Sieving mechanism based on:** **Charge-to-mass ratio** **Mass**	**peptides, proteins, DNA**

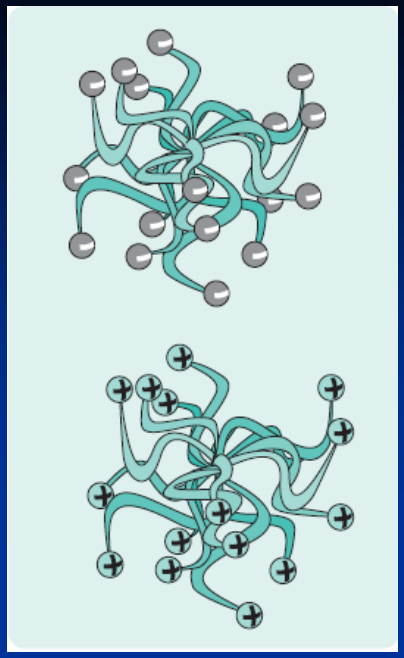
As a rule, the different modes can be accessed simply by altering the buffer composition.

Capillary zone electrophoresis

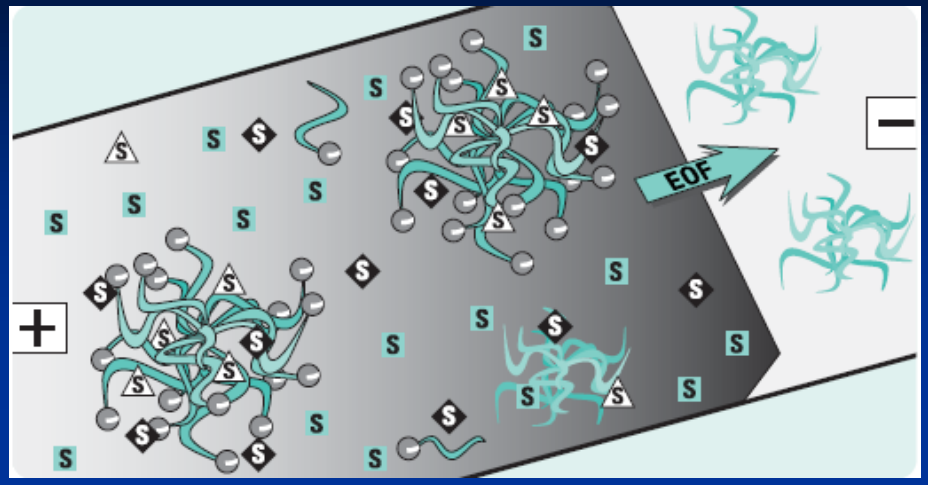


A capillary is filled with an electrolyte (run buffer),
the sample is introduced at the inlet
and the electrical field is applied.

Separation occurs because solutes migrate at different velocities and in discrete zones.



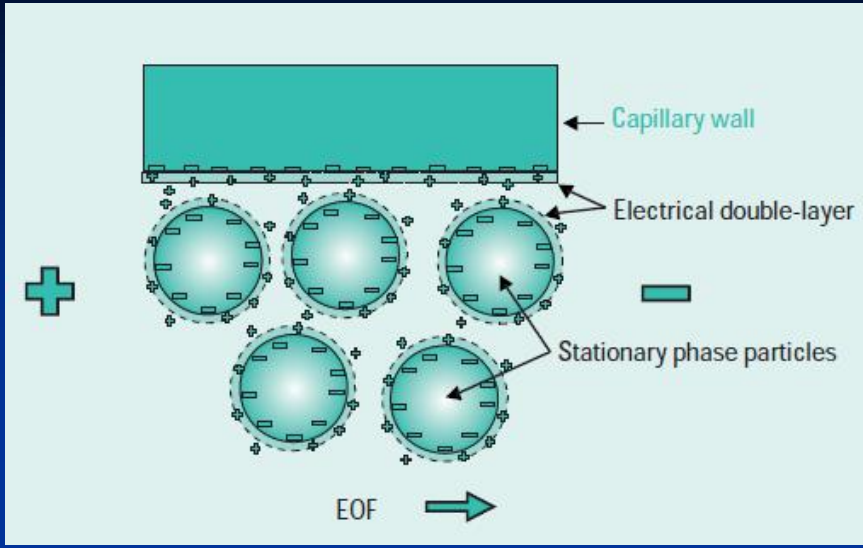
Micellar electrokinetic chromatography



It is a hybrid of electrophoresis and chromatography
It is the only electrophoretic technique that can be used for the separation of neutral solutes as well as charged ones.

The analytes partition between the micelles and the running through both hydrophobic and electrostatic interactions. The micelles act as a stationary phase like in chromatography. But this stationary phase is moving!
For neutral species, only partitioning in and out of the micelle effects the separation.

Capillary electrochromatography



It is a form of miniaturized liquid chromatography, which uses an electric field to pump liquid (EOF) through a packed chromatography column.

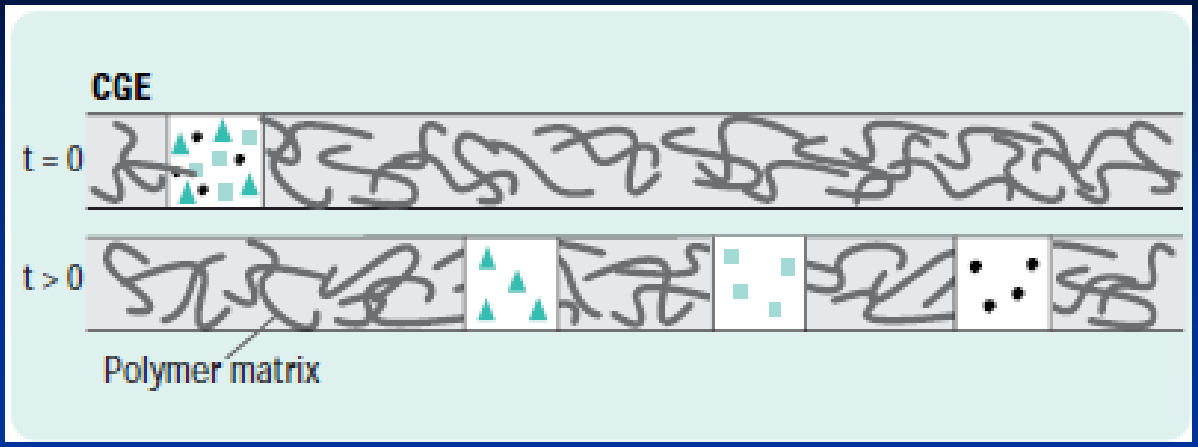
Stationary phase packed in a fused silica capillary, with an ID of 50-200 μm, is used to obtain separation. HPLC type silica based **reversed phase particles**, 1-5 μm, are mainly used as a stationary phase.

If the solutes are uncharged, separation is obtained by differential partitioning of the solutes between mobile and stationary phase.

If the solutes are charged, they will have an additional, electrophoretic velocity component.

In order to avoid outgassing and bubble formation in the column at high currents, buffer vials on inlet and outlet side are pressurized at 2-12 bar.

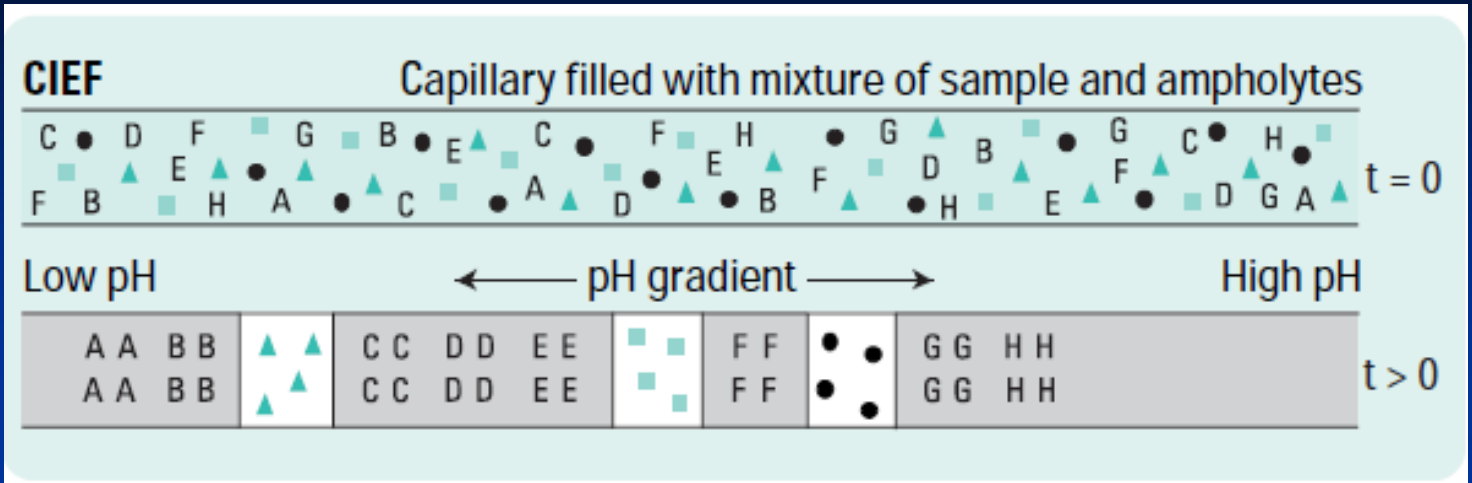
Capillary gel electrophoresis



The size-based separation of macromolecules such as proteins and nucleic acids. Macromolecules such as DNA and SDS-saturated proteins cannot be separated without a gel since they have invariable mass-to-charge ratios.

To separate proteins according to size, they have to be denatured with SDS. The size separation is then obtained by electrophoresis of the solutes through a suitable polymer, which acts as a "molecular sieve".

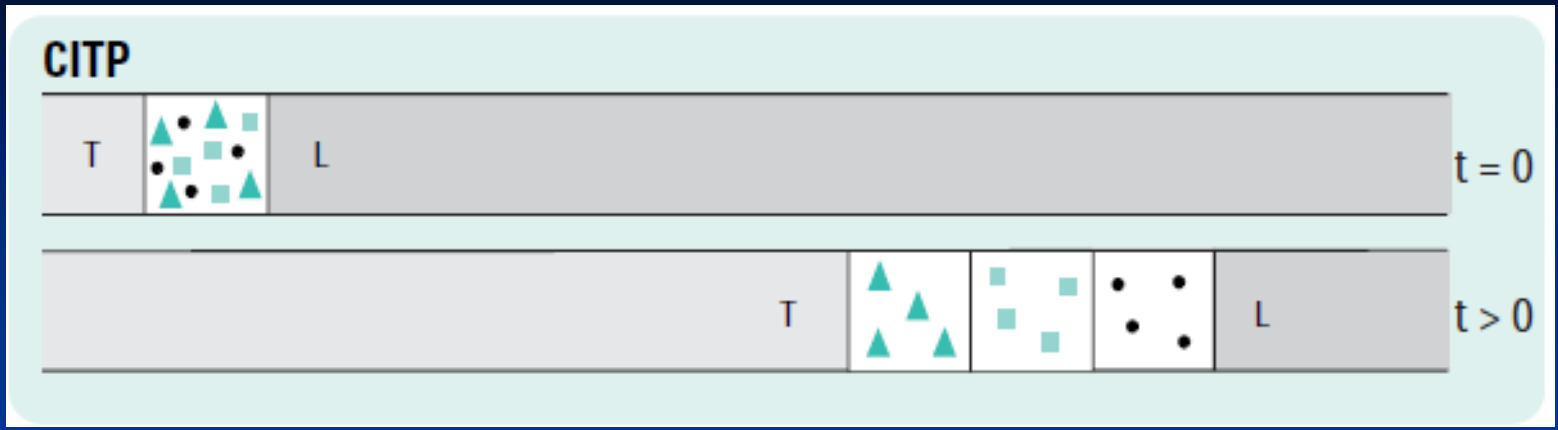
Capillary isoelectric focusing



It is a "high resolution" electrophoretic technique used to separate peptides and proteins on the basis of their isoelectric point (pI).

pH gradient is formed within the capillary using ampholytes. Ampholytes are zwitterionic molecules that contain both an acidic and a basic moiety and can have pI values that span the desired pH range. After filling the capillary with a mixture of solute and ampholytes, the pH gradient is formed. With a basic solution at the cathode and an acidic solution at the anode, upon application of the electric field the charged ampholytes and proteins migrate through the medium until they reach a region where they become uncharged (at their pI). This process is known as "focusing".

Capillary isotachophoresis



T terminating electrolyte, L leading electrolyte

It is a "moving boundary" electrophoretic technique.
A combination of two buffer systems is used to create a state in which the solutes all move as connected, separate bands and at the same velocity. The zones remain sandwiched between so-called leading and terminating electrolytes.

For anion analyses: the leading electrolyte contains an anion with the highest effective mobility among the anionic solutes. The terminating anion must have the lowest mobility. In the electric field the anions start to migrate towards the anode. The leading anion moves fastest, followed by the anion with the next highest mobility, and so on. Finally, the individual anions migrate in joined zones, but all move at the same velocity, as defined by the velocity of the leading anion.

Chromatography and clinical analysis

Accurate clinical diagnosis often requires detection and measurement of biological analytes in body fluids such as:

- serum/plasma,
- urine,
- cerebrospinal fluid,
- saliva.

Capillary electrophoresis

Saliva analysis: simultaneous determination of the ions NH^{4+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}

Cost-effective DNA sequencing and genotyping that provides high throughput and high accuracy sequencing information.

Forensic science

Methods of DNA detection for PCR-amplified DNA fragments,

Generation of profile from highly polymorphic genetic markers which differ between individuals.

Detection of specific mRNA fragments to help identify the biological fluid or tissue origin of a forensic sample.