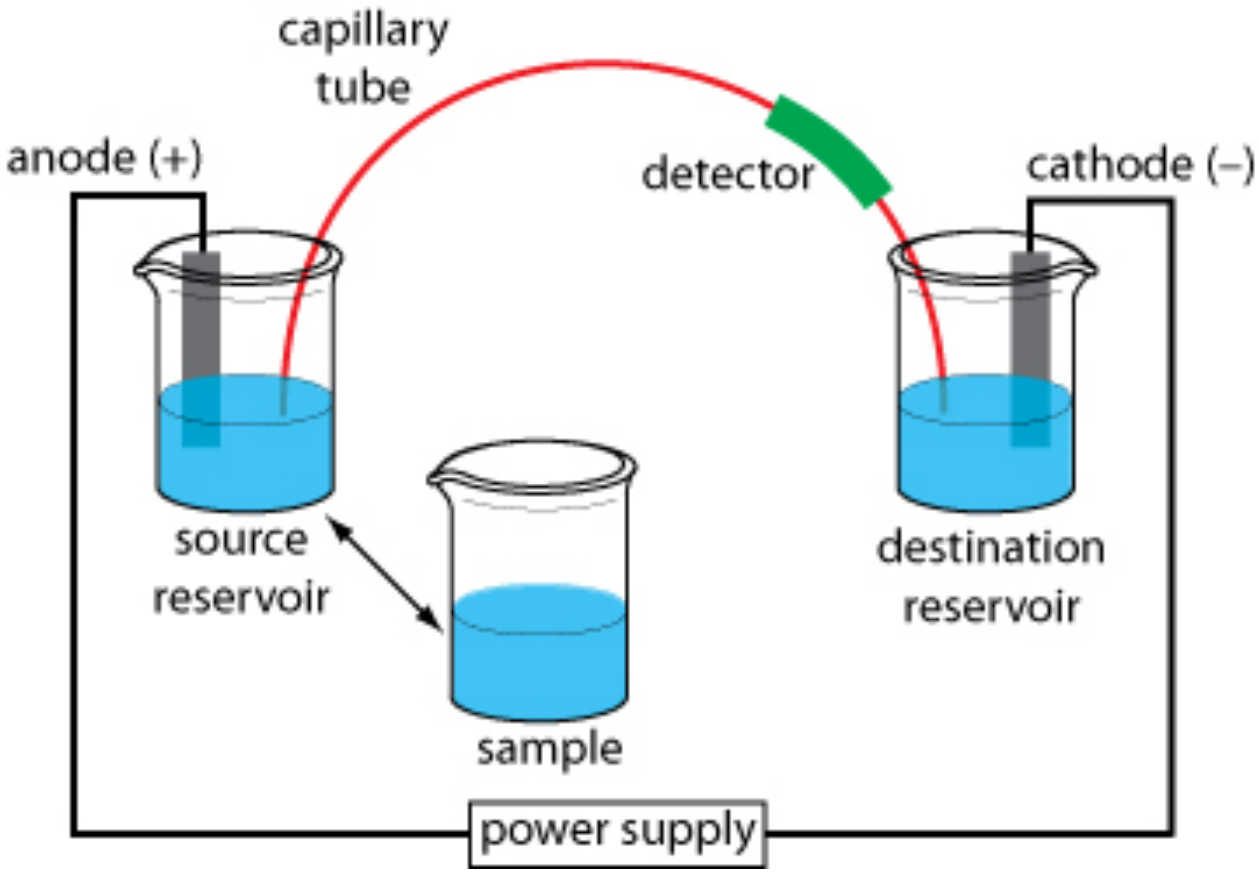
CAPILLARY ELECTROPHORESIS

Principi

- It separates charged molecules within an electric field.
- The fused silica capillary with a very small diameter, contains an appropriate buffer and with the 2 ends immersed in two separate tanks, containing two electrodes, responsible for generating the electric field



- Detector: uv or fluorescent. In the latter case the sample must be fluorescent or use a fluorescent marker.
- The sample is loaded by ΔP at one end of the capillary. **Advantages**

  Heat produced by the electric field dissipates quickly due to the favorable ratio between surface and volume. It is therefore possible to apply higher voltages than in traditional electrophoresis resulting in better separation and reduced analytical times

SEPARATION

The migration rate of the ion depends on the equilibrium between the thrust force of the electric field and the braking forces between ions and the surrounding medium. The potential gradient is:

$E=\Delta V/d$ d distance between electrodes, ΔV ddp

SEPARATION

Charged particles in the electric field is exposed to 2 forces, a thrust and a braking force

F_s=qE; F_f=$6\Pi\eta rv$ (η= *viscosity of the medium; r particle radius, v rate of migration)*

At the equilibrium the rate of migration is constant:

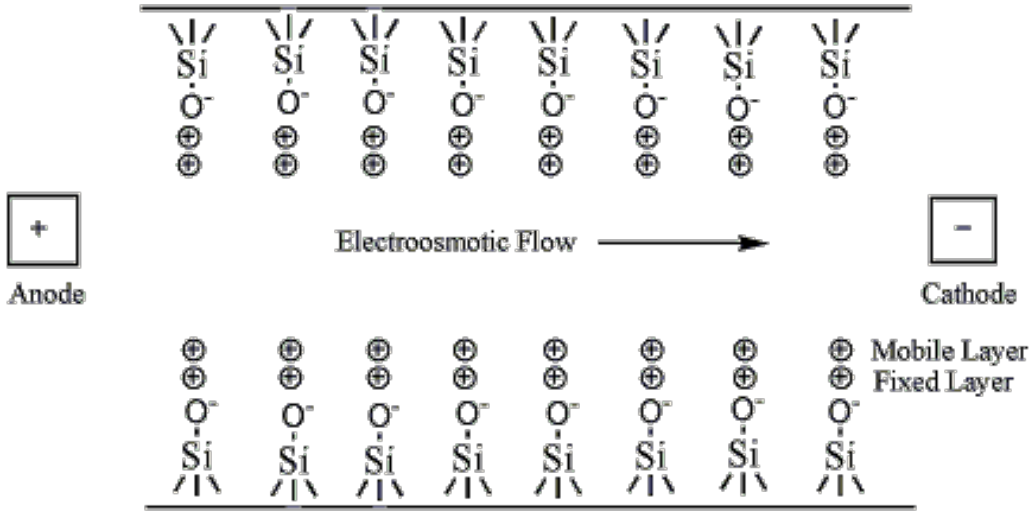
F_s=F_f, *therefore* qE= $6\Pi\eta rv$, *that is* $v= qE/6\Pi\eta r$, *but all particles are exposed to the same electric field and migrate in the same medium so* $K= E/6\Pi\eta$ *consequently* ***v=Kq/r,*** *therefore it is proportional to the ratio between particle charge and size. Larger particles weigh more so it is proportional to the charge / mass ratio.*

Electrophoretic mobility is dependent on:

- Size of the analyte molecule
- Buffer (type of buffer, concentration and pH)
- Temperature
- Electric field strength
- Support material
- pKa of the analyte molecule

It would seem that differently charged species follow an opposite direction, but this does not occur due to the phenomenon of ***ELECTROSMOSIS.***

1. In a fused-silica capillary, silanol (Si-OH) groups attached to the interior wall of the capillary are ionized to negatively charged silanoate (Si-O−) groups at pH values greater than 3.



2. This causes an increment of the positive potential as you approach the walls and causes the water molecules in the buffer to orient themselves accordingly..
3. The mobile cation layer is pulled in the direction of the negatively charged cathode when an electric field is applied. Since these cations are solvated, the bulk buffer solution migrates with the mobile layer, causing the electroosmotic flow of the buffer solution. The rate of EOF is dependent on the field strength and the charge density of the capillary wall. The wall's charge density is proportional to the pH of the buffer solution.

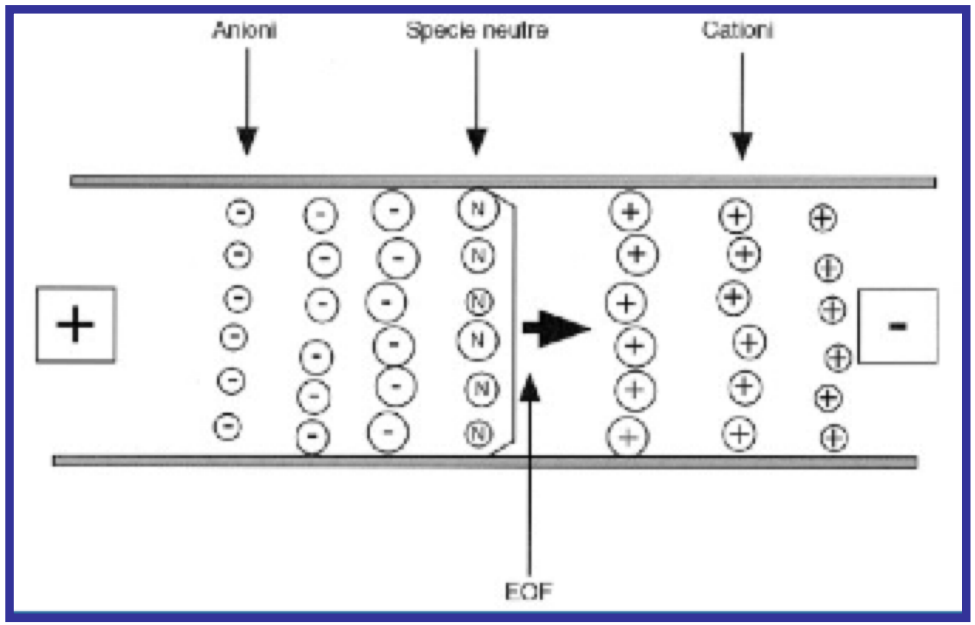
The existence of electrosmotic flow implies that all species, regardless of their charge, migrate to the cathode.

The migration speed for the different species is as follows:

- Cations: ionic mobility + EOF
- Neutral compounds: EOF
- Anions: EOF - ionic mobility

The rate of EOF exceeds the rate of anions (without EOF in theory) of about 10 times.

Examples of separtaions



Variables influencing EOF

1. Buffer pH.

Buffer strength.

3. Electric field
4. Temperature

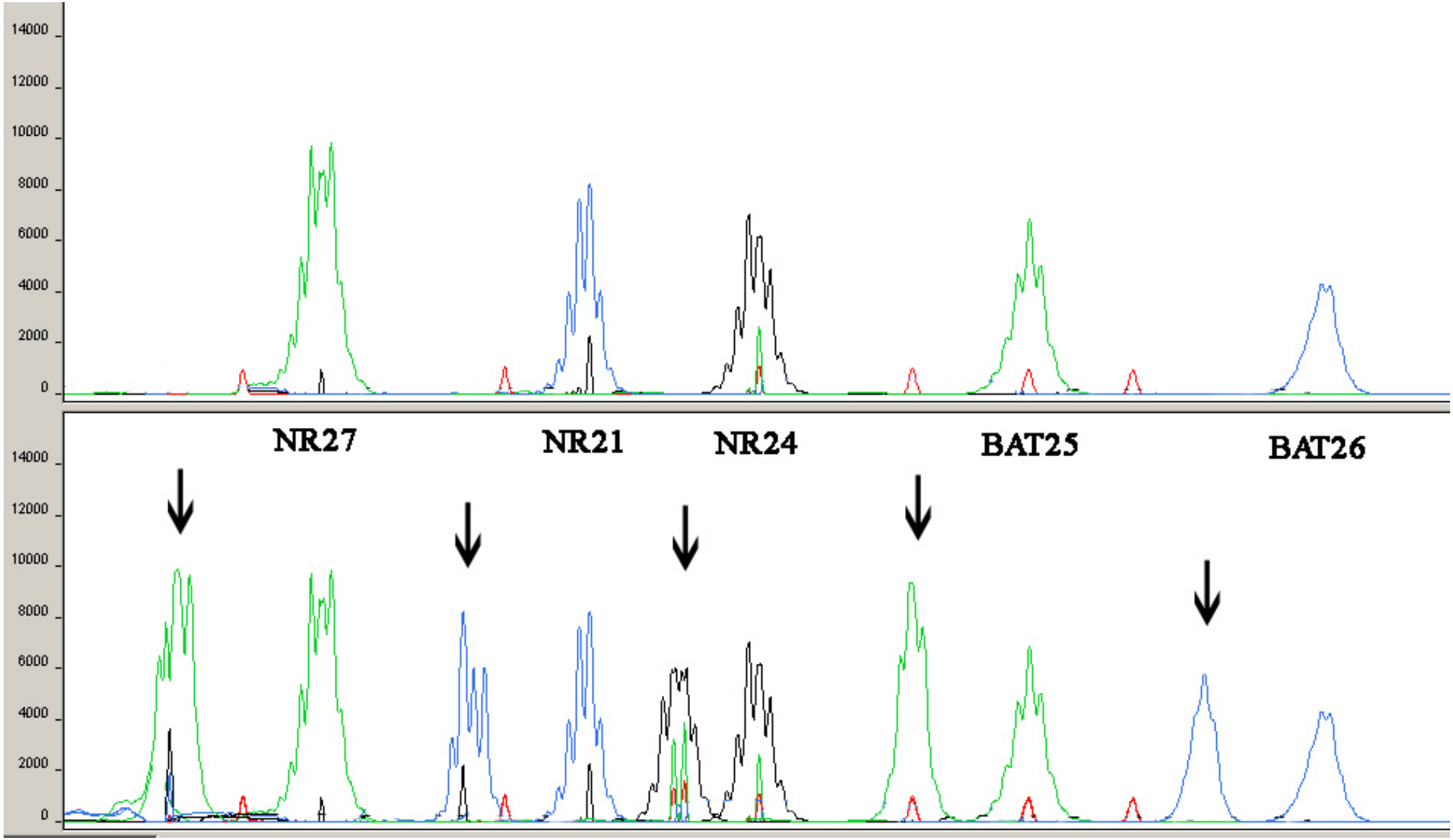
Molecules separted by CE

- aa
- peptides
- proteins
- nucleic acids
- inorganic anions
- organic acids and bases
- cells

ADVANTAGES OF CE

- high separation efficiency
- small amount of sample
- rapid separation
- selectivity
- automation
- possibility of quantification
- reproducibility
- possibility of coupling with mass spectrometer

Examples: MSI in Colorectal cancers



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