# Chiral analysis by using Capillary Electrophoresis

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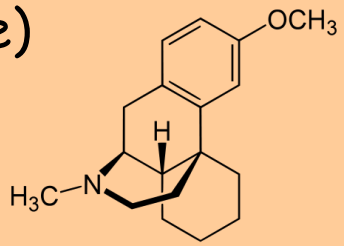
# Chiral analysis in forensic toxicology: why?

Enantiomers of drugs have often a different activity:

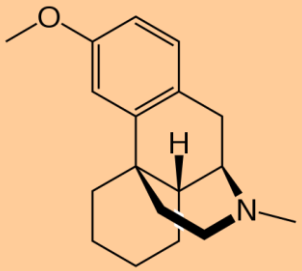
e.g. dextromethorphan (OTC antitussive)




and levomethorphan.

(the so called synthetic heroin,

scheduled as narcotic)



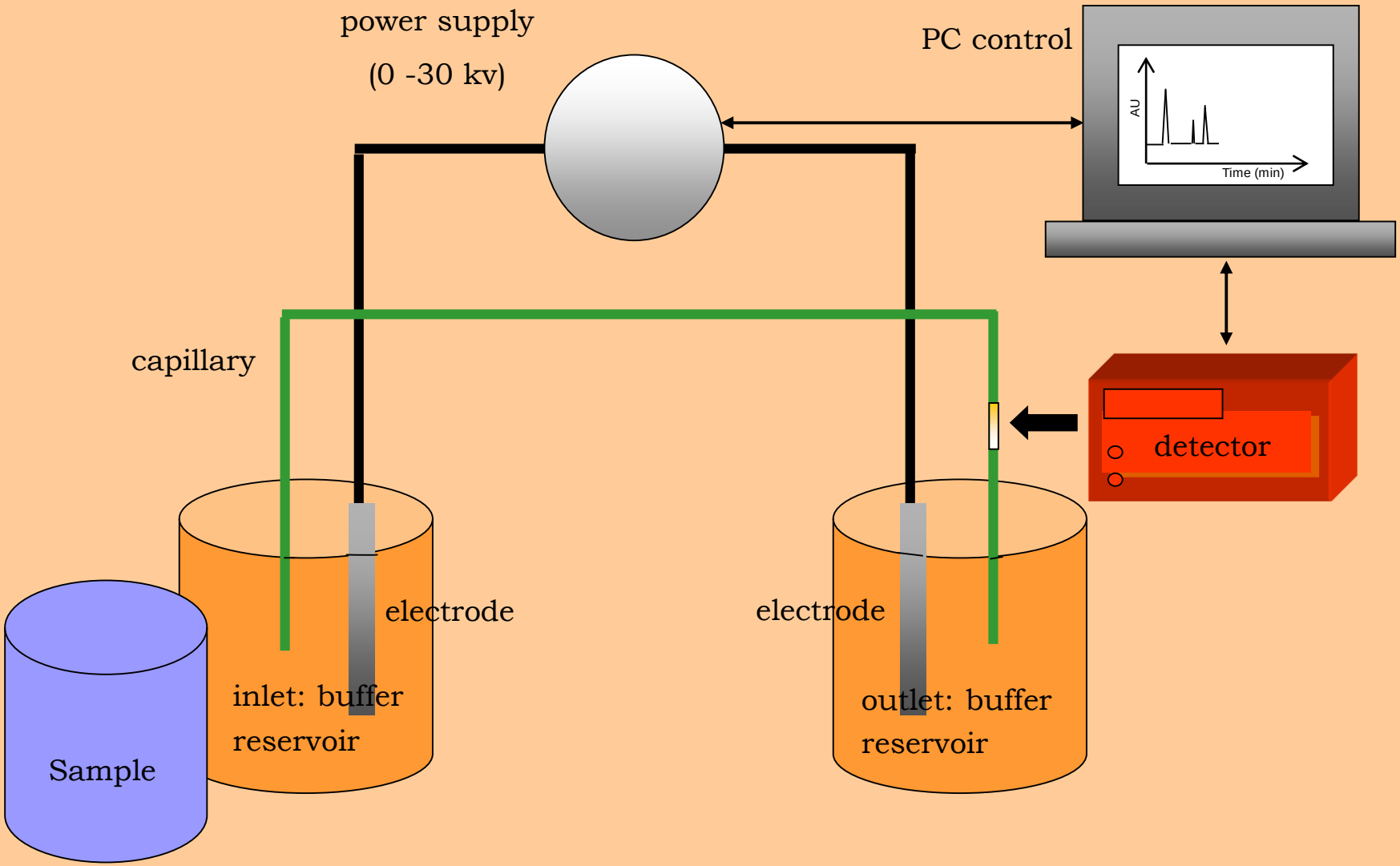
# Chiral analysis in forensic toxicology: why?

- Enantiomers of drugs produce different metabolites with potentially different activities and kinetics (e.g. amphetamines)
- Enantiomers of drugs (and metabolites) react differently with antibodies and may produce artifacts in immunoassays
- The presence of an enantiomerc abundance difference between parent drug and the drug present in a biological sample indicates that the drug has been metabolized, excluding contamination.
- The chiral analysis of a drug may give information on the synthesis process (e.g. amphetamines)
- The chiral analysis of a drug may prove its synthetic or natural origin (e.g. *l*-cocaine: natural; racemic cocaine: synthetic)

# Chiral analysis: approaches

- Cristallization
- Gas-chromatography
  - Formation of diastereomers
  - Chiral columns
- Liquid-chromatography
  - Formation of diastereomers
  - Chiral columns
  - Chiral selectors in the mobile phase
- Capillary electrophorresis
  - Formation of diastereomers
  - Chiral selectors in the buffer electrolyte
  - Chiral MEKC

# Typical CE instrumental set-up



# Characteristics of CE

- separation takes place in narrow-bore (20-100 μm i.d., 30-100 cm long) fused silica capillaries
- high electric resistance and heat dispersion → high voltages (up to 30 kV) → high electric fields (up to 1,000 V/cm)
- high separation efficiency (>> $10^5$ plates)
- high mass sensitivity (pg with UV detectors)
- small sample volumes injected (nl)
- possibility of coupling different separation modes
- *"in column"* optical detection

# Advantages of CE

- possibility of interfacing with different detection techniques (UV-Vis, FL, EC, MS etc)
- different modes of separation to vary selectivity
- versatility of application (from inorganic ions to large DNA fragments)
- minimum consuption of solvents, samples
- ruggedness, simplicity, low time and manpower consume
- automated instrumentation

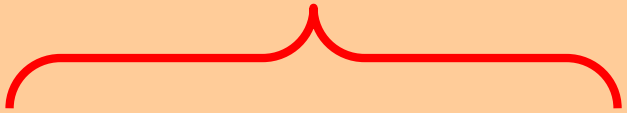
# Fields of application

- Inorganic ions
- Organic acids, bases
- Drugs
- Enantiomeric compounds
- Amino acids, Peptides, Proteins
- Nucleic Acids and their components
- Intact cell, bacteria, viruses
- Enzyme activities
- Drug-to-protein interactions

...

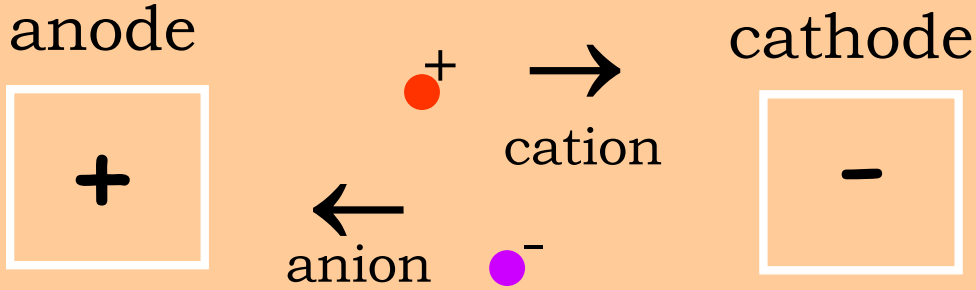
# Electrophoretic Separation: principles

In a solution, under the influence of an electric field,
species with different charges and/or sizes migrate
at different velocities depending upon their mobility.
If two analytes exhibit different mobilities,
in principle, they can be separated by CE.



Electrophoretic mobility + Electroosmosis

# Electrophoretic velocity (v)




$$F_{electrostatic} = E \cdot q$$

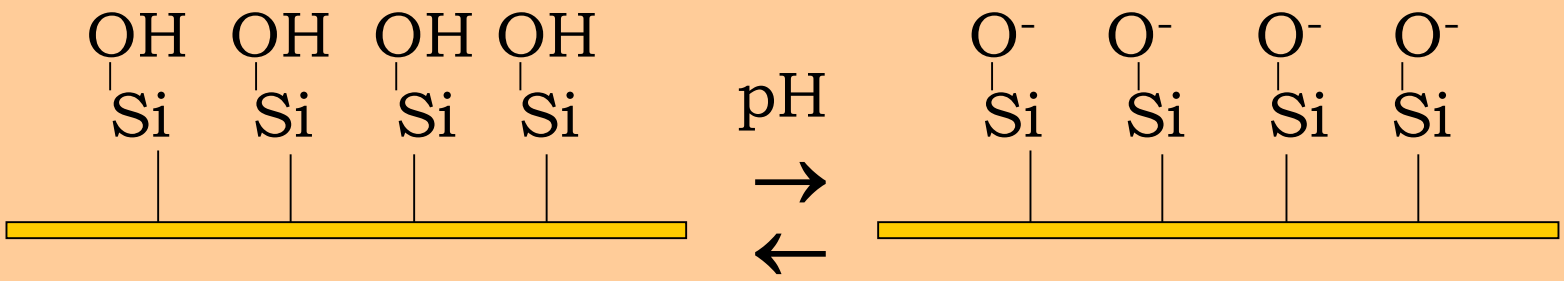
$$F_{viscous} = - 6\pi \cdot \eta \cdot r \cdot v$$

$$F_{electrostatic} = - F_{viscous}$$

$$E \cdot q = 6\pi \cdot \eta \cdot r \cdot v$$

$$\boxed{v = E \cdot q / 6\pi \cdot \eta \cdot r}$$

# Electroosmotic flow

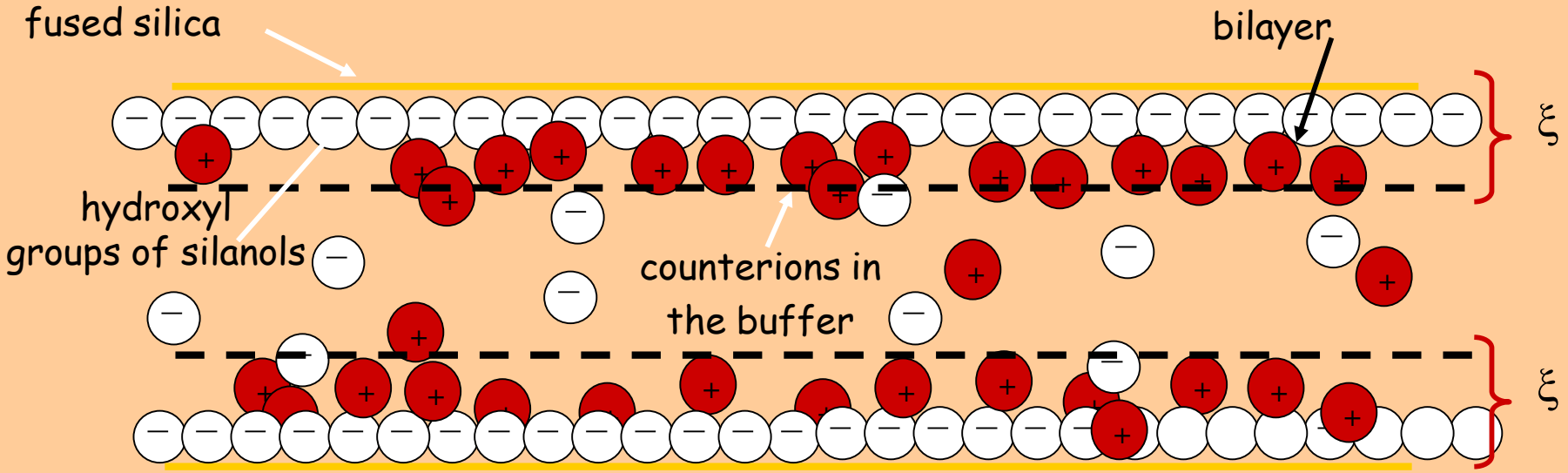



EOF is inherent in any electrophoretic situation in slab-gels or silica capillaries

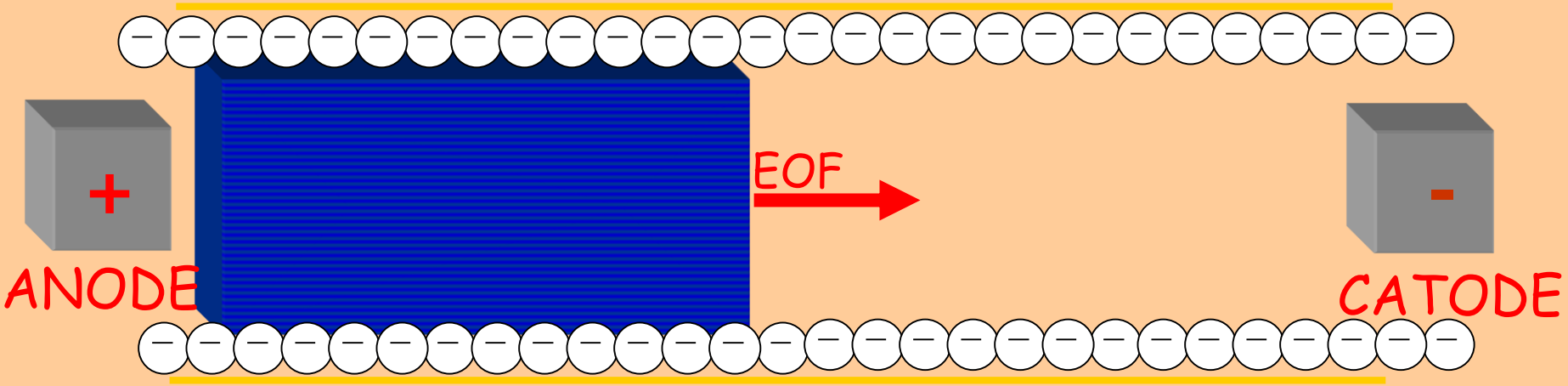
$$\mu_{eof} = \varepsilon \cdot \xi / 4\pi\eta$$

| | | |
|---|---|---|
| $\xi$ | = | zeta potential (determined by the surface charge on the capillary wall (pH, ionic strenght, ion-pair, additives...) |
| $\eta$ | = | buffer viscosity |
| $\varepsilon$ | = | buffer dielectric costant |

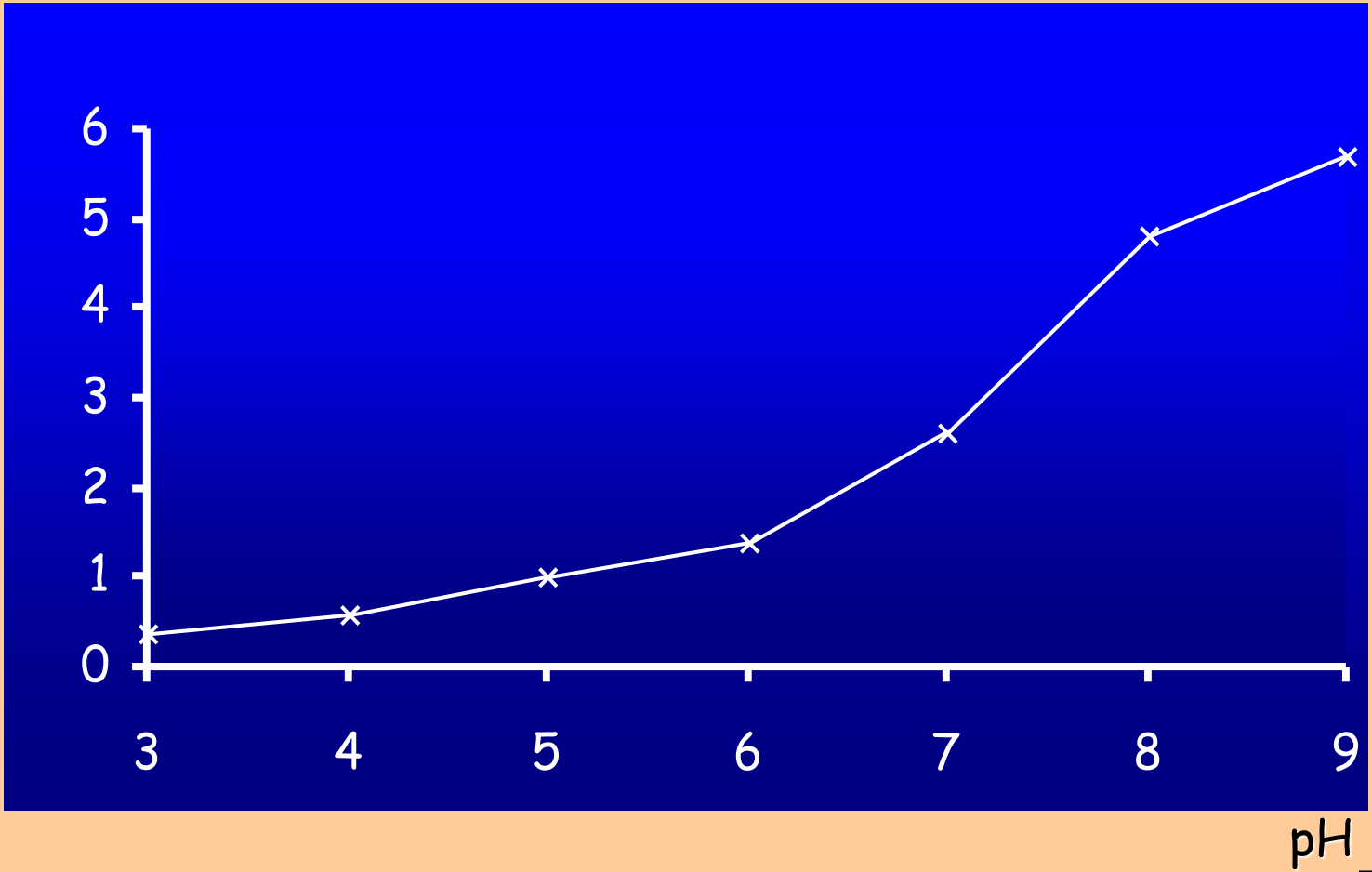
# Electroosmotic flow (EOF)




ξ = zeta potential : the change in potential across a double layer



# Dependence of EOF on pH




As pH increases, charge increases and $\mu_{eof}$ increases

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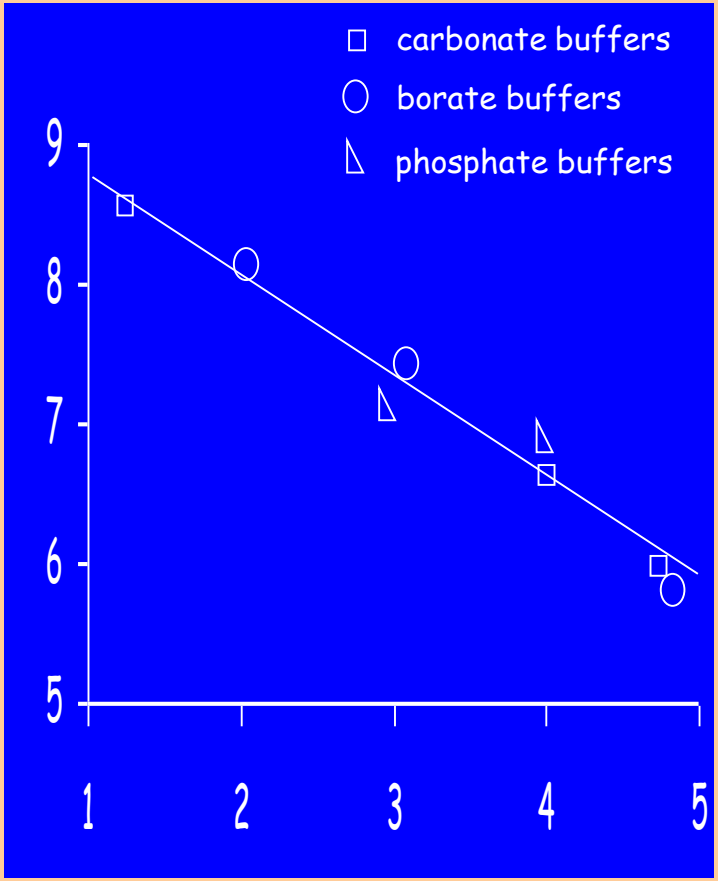
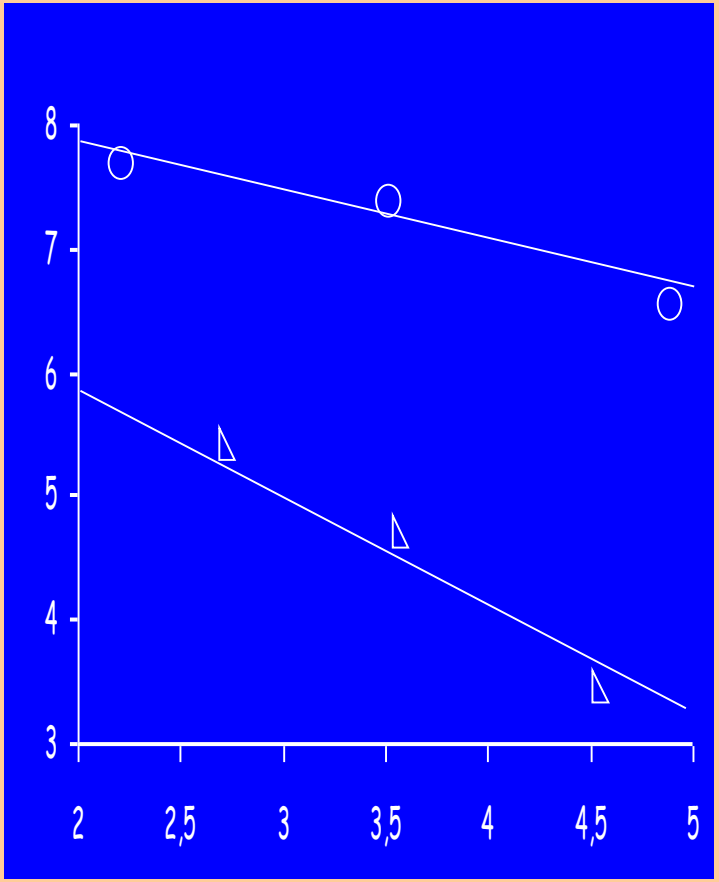
# Dependence of EOF on ionic strength




As the ionic strength and the concentration of the buffer increase the thickness of the layer decreases and $\mu_{eof}$ decreases.

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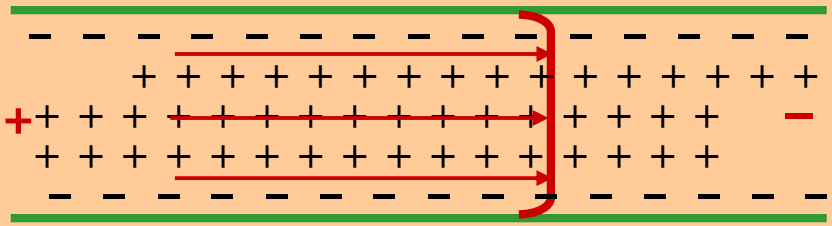
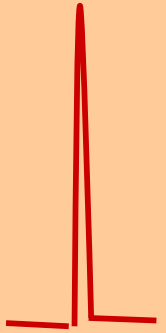
# Effect of flow profiles on zone width

Cross-sectional flow profile

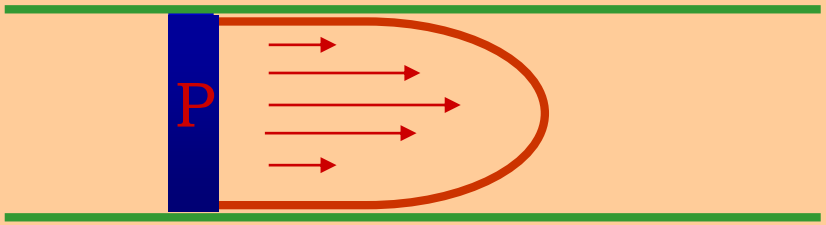
generated by electroosmotic flow

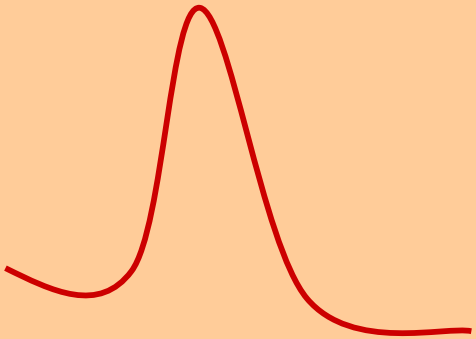




Cross-sectional flow profile

generated by hydrodynamic flow



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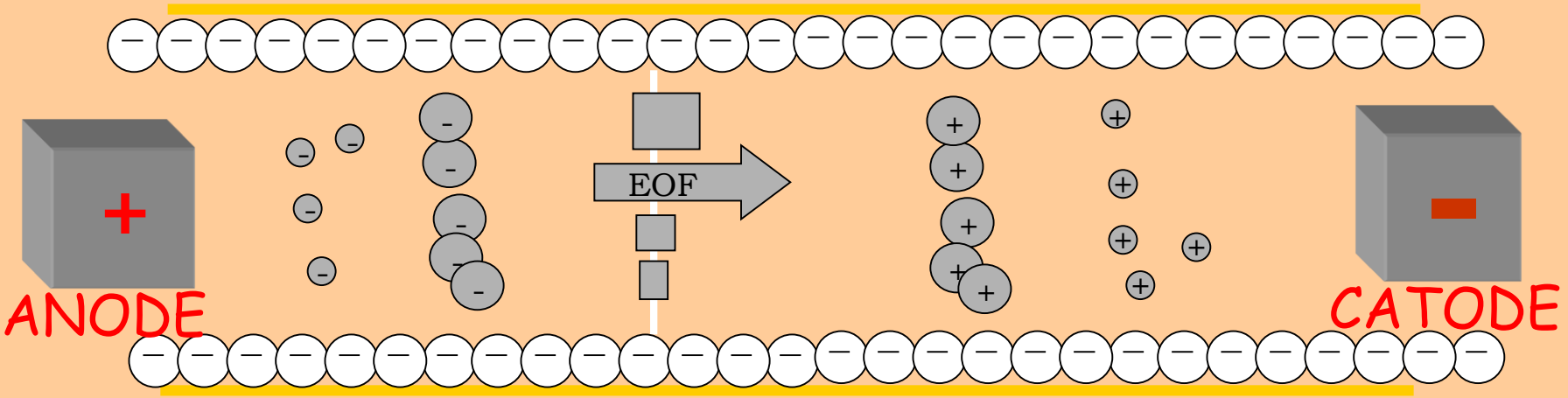
# Ion velocity and apparent velocity

$$V_{app} = V_i + V_{eof}$$

For any given ionic solute the apparent velocity is a combination of the effective velocity and the velocity of the EOF

# Electrophoretic Separation

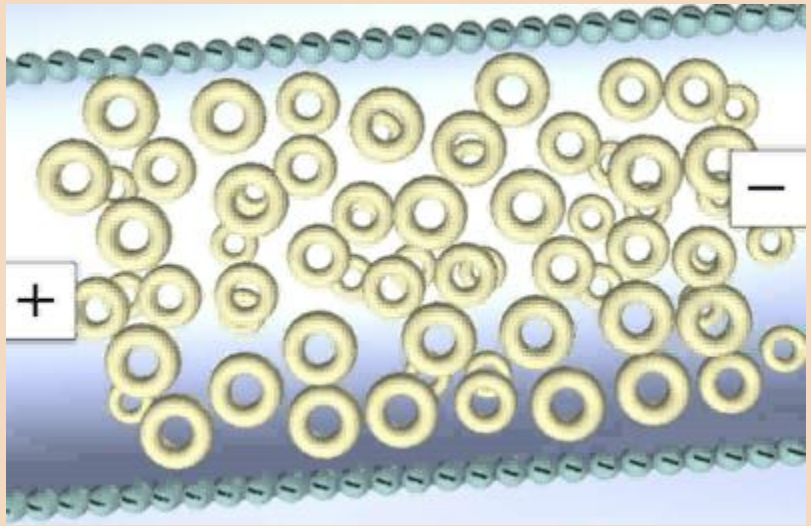
When the voltage is applied the anion electrophoretic migration will be toward the anode and cation electrophoretic migration will be toward the cathode.
The endoosmotic flow will be in the direction of the cathode.




The mobility of the EOF is usually greater than the mobilities of the ions, meaning that positive, neutral and negative analytes can be detected in the same run:

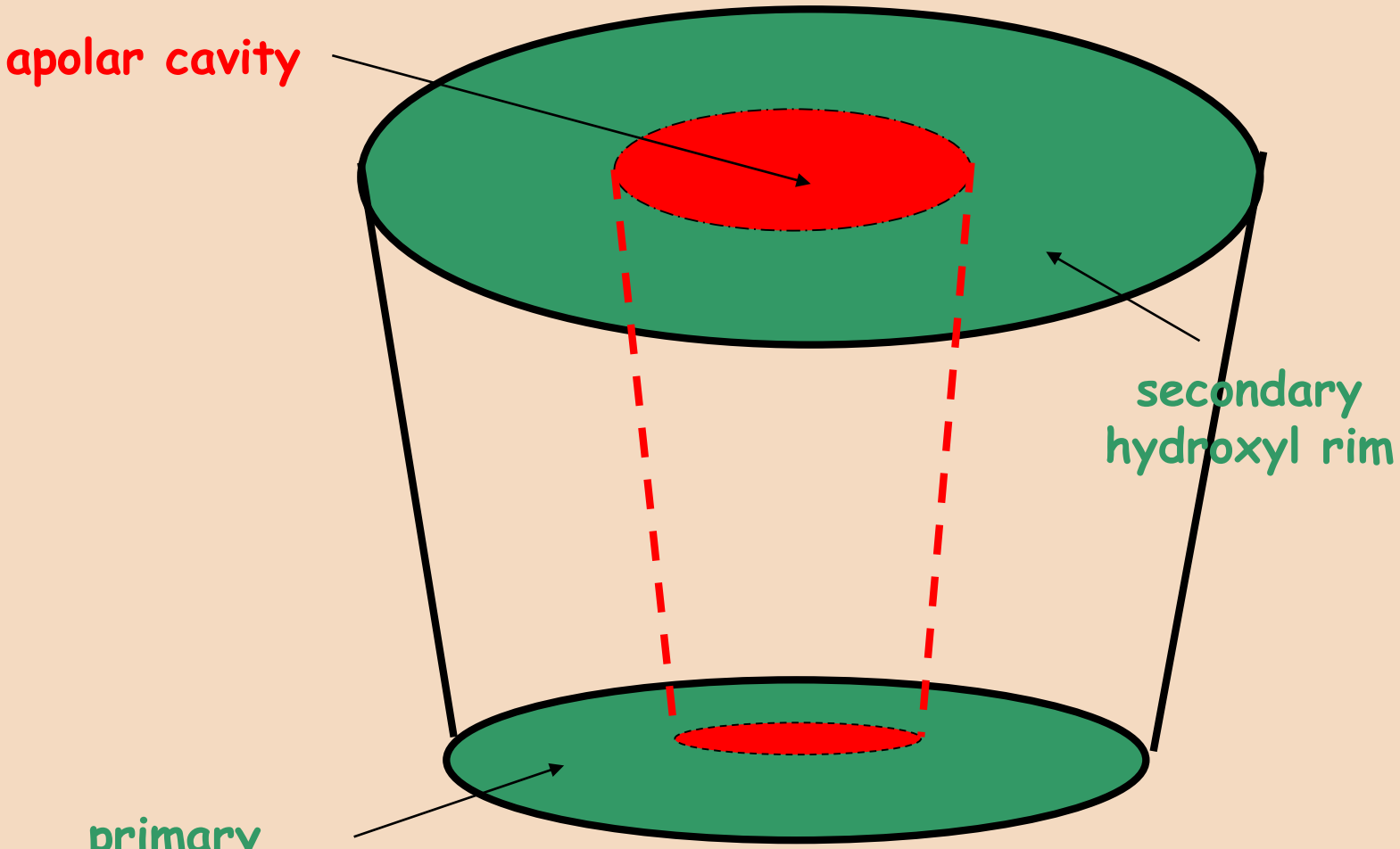
| | | |
|---|---|---|
| cations (+) | $(v_i > v_{eof})$ | $v = v_{eof} + v_i$ |
| neutral ions | $(v_i = v_{eof})$ | $v = v_{eof}$ |
| anions (-) | $(v_i < v_{eof})$ | $v = v_{eof} - \mu_i$ |

# Chiral separations complex formation electrophoresis

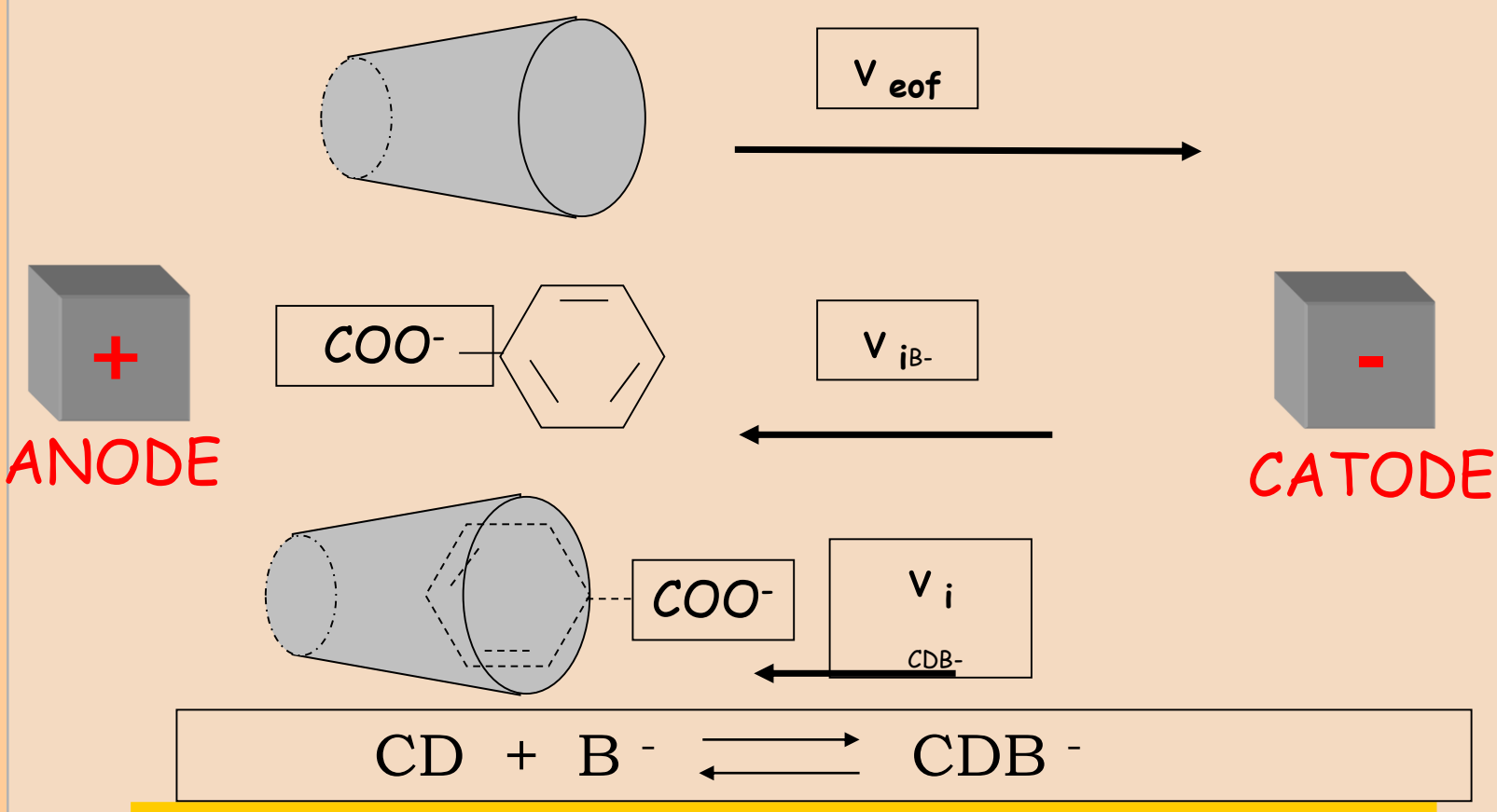


# Chiral separations in CE

Ciclodextrin (CD scheme)



# Chiral separations in CE

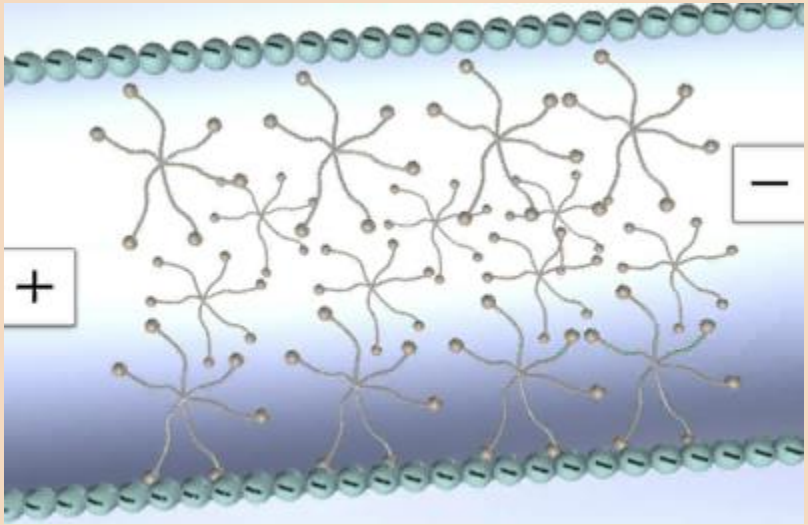


# Chiral separations in MEKC

1. Use of chiral surfactants:
   bile salts in neutral and alkaline conditions;
   taurine coniugates of bile salts in acidic conditions;
   amino-acids derived surfactants;

2. Use of chiral additives:
   $\alpha$, $\beta$, $\gamma$ CDs native and derivatized;
   crown ethers;
   copper (II)-aspartate;
   antibiotics…

# Micellar electrokinetic capillary chromatography (MEKC)

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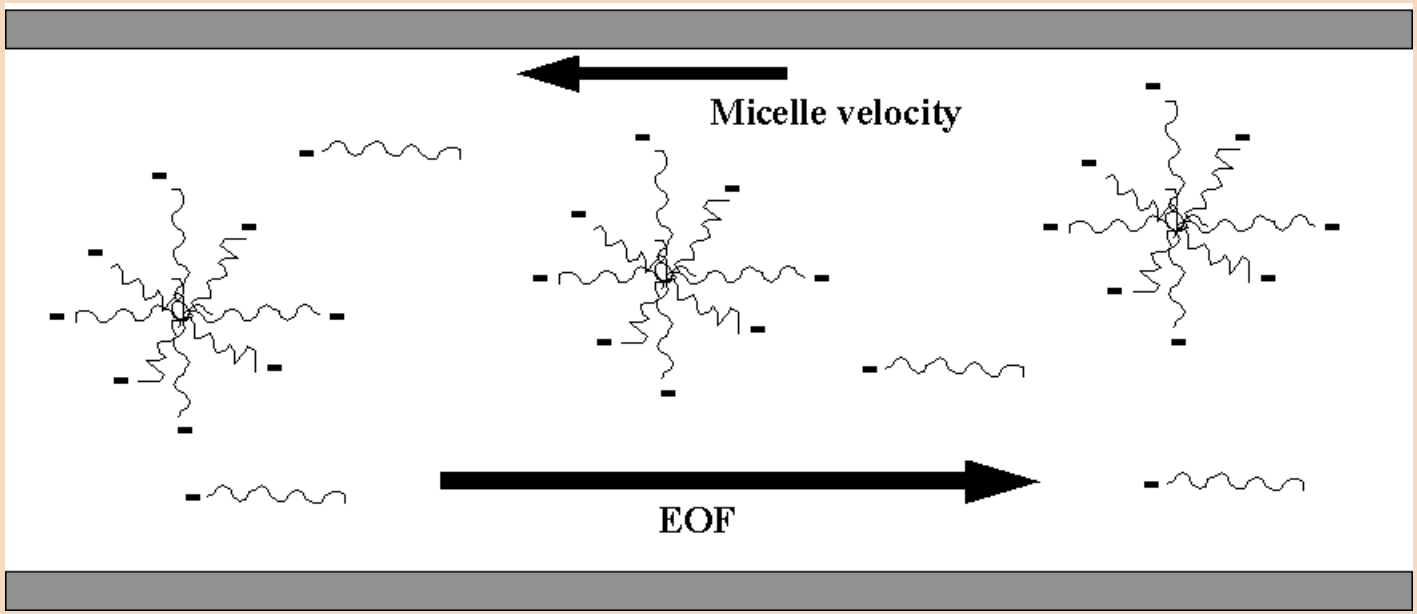
# SDS micelles in the run buffer

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# MEKC

- Used to separate neutral compounds
- Selectivity is based on differences in hydrophobicity
- Migration time is dependent on analyte-micelle interaction
- Selectivity "similar" to RP-HPLC for neutral molecules

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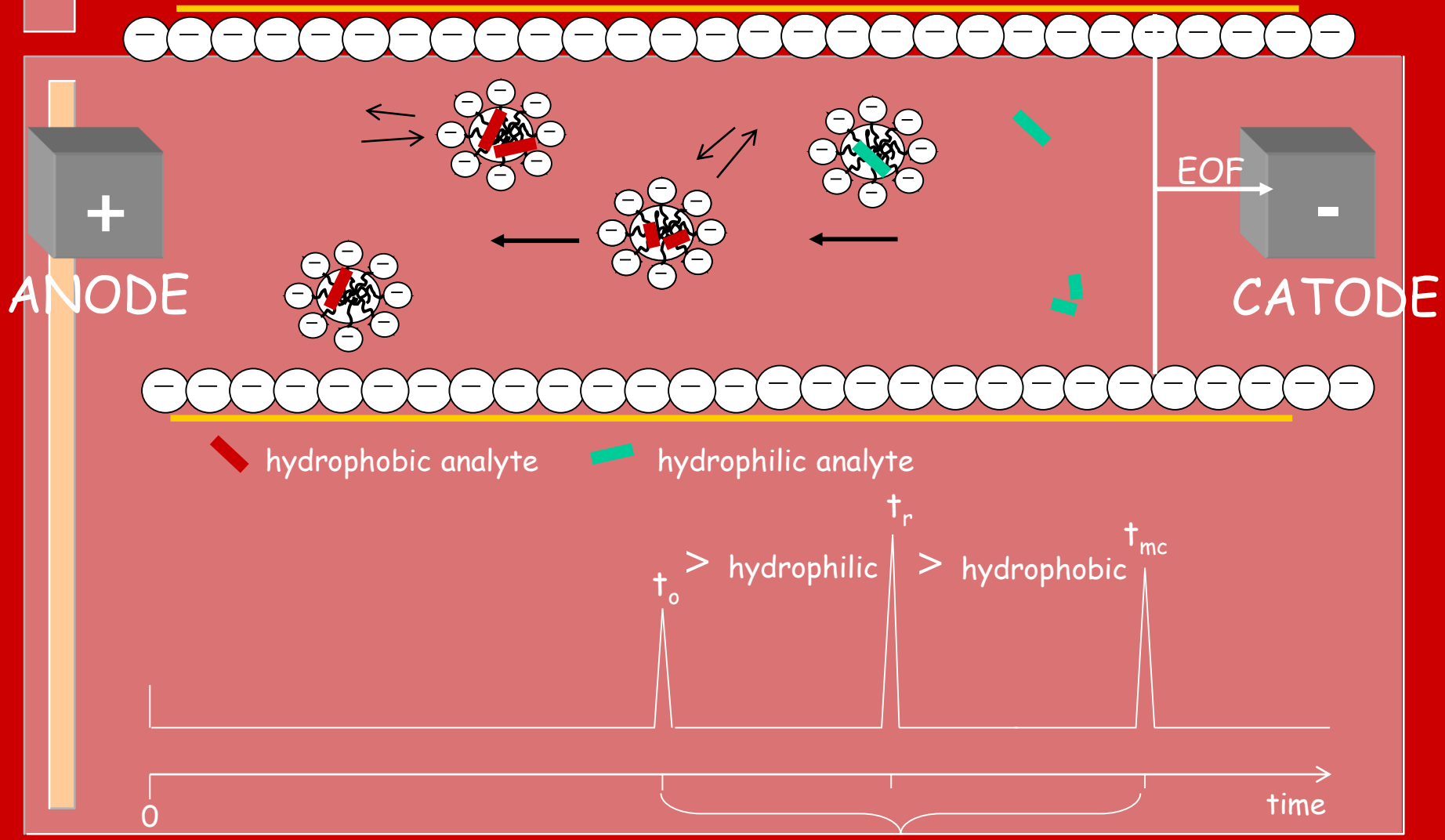
# Scheme of MEKC separation




Time window

# Separation according to CD-MEKC