Capillary electromigration techniques:

EKC: MEKC, MEEKC
CEC
CITP
CIEF
Electrokinetic Chromatography (EKC)

A separation technique based on a combination of electrophoresis (buffered electrolyte solutions) and interactions of the analytes with additives (e.g. cyclodextrins, surfactants), which form a dispersed phase moving at a different velocity than the analytes.

In order to achieve separation either 1) the analytes or 2) the dispersed phase (=secondary phase) should have charges.

Notes:
1. EKC is not totally compatible with the definition of chromatography.
2. The same technique is also called electrokinetic capillary chromatography (ECC)
Electrokinetic chromatography is a new type of analytical separation method, which utilizes the experimental technique of capillary zone electrophoresis in combination with the principle of chromatography. A theoretical plate number of 250,000 is easily obtained in 10 min.
The capacity factor \( k' \) of the analyte (solute, studied compound) can be calculated from retention times

\[
k' = \frac{(t_R - t_0)}{(1 - t_R/t_c)} t_0
\]

**Cyclodextrin-induced electokinetic chromatography (CD-EKC)**

5-12.5 mM carboxymethyl cyclodextrin in 25 mM borate, 1 mM 1-bromo-4-naphtalenesulonate (BrN), pH 8-5

**Sudan III** a lysochrome (fat-soluble dye) diazo dye.

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**EKC: Chiral separation in capillary electrophoresis**

The chemicals for chiral separation are used as additives in the electrolyte solution. They can be divided into 3 main groups:

1. **Inclusion systems** (e.g. cyclodextrins and crown ethers),
2. **Enantiomer selective** metal-ion complexes (copper(II)-L-histidine or copper(II)-aspartame) and
3. **Optically active surfactants** (e.g. chiral mix-micelles or bile acids).

Those enantiomers which interact the most intensively with cyclodextrin cavity, move with the lowers speed.

Usually **cyclodextrins** are added to **acidic electrolyte solutions (BGE)**. **But, they** can also be added into **alkaline electrolyte solutions**. Then the enantiomers are anions and their apparent mobility (natural movement) is from detector to capillary inlet. In this case, the enantiomer that interacts the most with cyclodextrin enters first the detector, since its migration (speed) away from the detector is hindered the most.

**Crown ethers** (e.g. **18-crown-6 ether**) are suitable for chiral separation of primary amines.
**Cyclodextrin - chiral selector**

Cyclodextrins (sugars) form cavity-like structures which interact with anionic and non-ionic compounds.

Mechanism: adsorption-partition procedure during separation

The more efficiently the compound is moved into the cavity, the more it is retained and the later it is moved to detection.

Enantiomers have different geometrical structures: The other form moves more deeper into the cavity than the other -> this causes separation of the compounds, because their electrophoretic mobilities are different.

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**Monomers of chiral selectors: Charged Cyclodextrins**

- **β-Cyclodextrin**
- **CM-β-CD (ds 1)**
- **H-(DMS)-β-CD**
- **H-(DAS)-β-CD**
- **H-(DMCM)-β-CD**
Chiral selectors:
Calixarene

Fig. 1: 1,3,5-OCH₃-2,4,6-OCH₃-R=p-tolyl/calix[6]arene (L_{COOH}, L_{H₂}, R=CONHOH; R=COOH; L_{H₂}, R=CONHOH)

Chiral selectors:
Vancomycin

Vancomycin is an glycopeptide antibiotic

MW 1449 g/mol
Chiral separation in CE

Epinephrine-catecholamine

Choice of chiral selector for enantioseparation by capillary electrophoresis
Blanco M. Valverde I.
TrAC Trends in Analytical Chemistry, Volume 22, Number 7, July 2003, pp. 428-439(12)

2-Phenyl-propionic acid

2.5. Conditions: PACE System MDQ. Bare fused-silica capillary, 50 micrometers i.d. 20 cm to the detector, 31.5 cm total. 5% HS-γ-CD in 25 mM TEA Phosphate buffer, pH 2.5. Pressure injection, 0.3 psi for 4 seconds. Separation at 15 kV constant voltage, 22 degrees C, malle at inlet. UV detection at 200 nm. Current 153 microamps.
Micellar Electrokinetic Chromatography (MEKC)

A special case of *electrokinetic chromatography*, in which the secondary phase is a micellar dispersed phase (pseudo stationary phase) in the capillary.

Note: The same technique is also called micellar electrokinetic capillary chromatography (MECC)

Micellar Electrokinetic Capillary Chromatography

*Micellar Electrokinetic Capillary Chromatography (MEKC or MECC)* is a mode of electrokinetic chromatography in which surfactants are added to the BGE (buffered electrolyte) solution at concentrations that form micelles.

The separation principle of MEKC is based on a differential partition between the micelle (a pseudo stationary phase) and the solvent (BGE). This principle can be employed with charged or neutral solutes and may involve stationary or mobile micelles.

MEKC has great utility in separating mixtures that contain both neutral and ionic species, and has become a valuable method in the separation of very hydrophobic pharmaceuticals from their very polar metabolites.

- Analytes moves into a micelle
- e.g. sodium dodecyl sulfate: polar headgroups, non-polar tails
Amphiphilic surfactant molecular structure

Hydrophobic (lipophilic) tail
Hydrophilic head
Micelle
Aqueous solution

Sodium dodecyl sulfate
Surfactant Tail
Surfactant Head
Surfactant Monomers
Micelle

celluloses

n = 10 (DDPO), 12 (DTPO) and 14 (DHPO).
Micellar electrokinetic capillary chromatography

- uses surfactants --> for micelle formation
- dynamic phases (fast interactions)
- hydrophilic and hydrophobic heads
- anionic and cationic
  e.g. SDS (sodium dodecy1 sulphate)
- selectivity with choice of surfactants

Micellar electrokinetic chromatography

- separation is based on distribution of the studied compounds between the electrolyte and the micelles--> electrophoretic mobility and hydrophobic interactions

- MEKC is a method for ionic and neutral compounds
Micellar electrokinetic capillary chromatography, MEKC
MEKC
-one or more surfactants are added to the electrolyte solution at concentrations which are above their critical micelle formation concentration (cmc)
-surfactants are amphiphilic compounds that have a carbon core with polar or ionic main group/functional group

-in aqueous solution surfactants form micelles when their concentrations are above their cmc

-micelles can also be formed on an oily droplet in aqueous solution (coating of the droplet) --> then the method is micellar microemulsion electrokinetic chromatography

MEKC

Surfactant or from a mixture of two or many surfactants
SDS, sodium salt of layryl sulphate
SDS and bile acids
etc.

-to get compounds separated, they should have different micelle-water distribution coefficients

-the flow of electrically neutral compounds in the electrolyte solution is to the same direction as that of electro-osmosis

-ionic and neutral compounds can be separated with the same MEKC technique

-the flow of the studied compounds is decreased, when the compounds interact with the micelles
Electrolyte solution: 3 mM 80/20 mol% POPC/PS in 50 mM CHES pH 9
Experimental: uncoated capillary 50/58.5 cm, 245 nm, 20 kV, inj. 5s 50 mbar, 25°C

**Electrophoresis 2009**
### Surfactants in MEKC

<table>
<thead>
<tr>
<th>Type</th>
<th>Surfactant</th>
<th>CMC (mM) in water</th>
<th>Aggregation number</th>
</tr>
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<tbody>
<tr>
<td>Anionic</td>
<td>SDS (Sodium dodecyl phosphate)</td>
<td>8.2</td>
<td>62</td>
</tr>
<tr>
<td>Cationic</td>
<td>DTAB (Cetyl trimethylammonium bromide)</td>
<td>14</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>CTAB</td>
<td>1.3</td>
<td>78</td>
</tr>
<tr>
<td>Non Ionic</td>
<td>Octylglucoside</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>n-Dodecyl-b-D-maltoside</td>
<td>0.16</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>Triton X-100</td>
<td>0.24</td>
<td>140</td>
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<tr>
<td>Zwitterionic</td>
<td>CHAPS</td>
<td>8</td>
<td>10</td>
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<tr>
<td></td>
<td>CHAPSO</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Bile Salt</td>
<td>Cholic acid</td>
<td>14</td>
<td>2 – 4</td>
</tr>
<tr>
<td></td>
<td>Deoxycholic acid</td>
<td>5</td>
<td>4 – 10</td>
</tr>
<tr>
<td></td>
<td>Taurocholic acid</td>
<td>10 –15</td>
<td>4</td>
</tr>
</tbody>
</table>

**The most popular surfactant in EKC**

*SDS sodium dodecyl sulphate*

\[
k = \frac{n_{mi}}{n_{BGE}}
\]

- \(n_{mi}\): Amount of the analytes (in mole) in micelle
- \(n_{BGE}\): Amount of the analytes (in mole) in BGE
MEKC (MECC) - principle

Basic operation:
High pH, SDS micelles, Positive polarity
Migration time of micelles (in micellar electrokinetic chromatography), $t_{mc}$
Experimentally determined as the migration time of a compound that is completely retained in the micellar phase

The neutrals move with EOF and are not separated.
Separation can only be realised by introduction of a chromatographic partitioning mechanism
5.2.2019

Separation in MEKC:
Add anionic or cationic surfactant, forming micelles with a hydrophobic core and hydrophilic shell.
Sodium dodecylsulfate (SDS) is the most common one.

Micellar Electrokinetic Capillary Chromatography

- The MEKC surfactants are surface active agents such as soap or synthetic detergents with polar and non-polar regions.
- At low concentration, the surfactants are evenly distributed
- At high concentration the surfactants form micelles. The most hydrophobic molecules will stay in the hydrophobic region on the surfactant micelle.
- Less hydrophobic molecules will partition less strongly into the micelle.
- Small polar molecules in the electrolyte move faster than molecules associated with the surfactant micelles.
- The voltage causes the negatively charged micelles to flow slower than the bulk flow (endo-osmotic flow).
Neutrals distribute (partition) between hydrophobic micelles (stationary phase) and background electrolyte (mobile phase).

But, the ‘stationary’ phase is not stationary (pseudostationary!)

MEKC

Partitioning of Analytes

- More the analyte is incorporated into micelle, the higher the electrophoretic mobility or more likely it will travel less slowly toward the cathode

The electropherogram is described in units of Intensity vs. Time (min)
MEKC with anionic micelles

Partitioning of neutral and ionic analytes

If the system contains both neutral and ionic species with micellar solution, the elution order is as follows;
I. More negatively charged anionic species
II. Less negatively charged anionic species
III. Neutral species (hydrophilic before hydrophobic)
IV. Less positively charged species
V. More positively charged species

Why is this?

MEKC

Partitioning of neutral and ionic analytes

• In CE the migration order of ionic species is cations first and anions last, but order is reversed in MEKC with anionic micelles due to the micellar interaction
• The negatively charged species are repelled by the anionic micelle while the positively charged species are attracted to the micelle; because the micelle elutes later than the buffer (EOF), so do the positively charged species which are attracted to it
• The more negatively charged species - more repelled by micelle than the less negatively charged species (elute earlier)
• The more positively charged species - more attracted (strong electrostatic interaction) to the micelle than the less positively charged species (elute later with the micelle)
• The neutral species migrate between the two extremes and their separation is based on hydrophobicity
Migration window for neutral solutes in MEKC

Elution window

Migration window

Solutes

EOF

Micelle

0 \ t_0 \ t_R_1 \ t_R_2 \ t_R_3 \ t_m \ Time

MEKC
Partitioning of Analytes

- Three types of interaction mechanisms known between micelle and analyte
  - Incorporation of analyte into hydrophobic core (usually highly hydrophobic and nonpolar analytes)
  - Adsorption of analyte on the surface layer
  - Incorporation of analyte as a cosurfactant

- Size, dipolarity and hydrogen bonding capabilities are important in contributing to the interaction and retention factor.
  - Solute molecule needs to be small enough to be accommodated by micelle
  - Solute hydrogen bond acceptor capabilities affect negatively the interaction

MEKC
Theory

- the retention factor is \( k \)
- \( n_{mc} \) is the amount of analyte incorporated in the micelle
- \( n_{aq} \) is the amount of analyte in the aqueous phase.
- \( t_R \) is the retention (migration) time of the analyte
- \( t_0 \) is the time it takes for the buffer alone to exit
- \( t_{mc} \) is the retention (migration) time of the micelle alone

\[
k = \frac{n_{mc}}{n_{aq}},
\]

\[
k = \frac{t_R - t_0}{t_0 (1 - t_R/t_m)}. \]

Remember in chromatography:

\[
K_D = k\beta
\]

From chromatogram:

- \( r_c \) is the column characteristic

- \( r_c = \) Column radius

- \( d_f = \) film thickness
MEKC
Theory

- Resolution equation

\[ R_t = \sqrt{N} \left( \frac{a - 1}{a} \right) \left( \frac{k_2}{1 + k_2} \right) \left( 1 - \left( \frac{t_0}{t_{nc}} \right) \right) \left( 1 + \left( \frac{t_0}{t_{nc}} \right) k_1 \right) \]

- \( \alpha = k_2/k_1 \)
- Last factor accounts for the effective capillary length which is different in MEKC (micelle is not stationary in capillary but migrates=length of micelle interacting with analyte in the capillary is shorter than the total distance the micelle migrates during the whole process—“migration time window”)
- The optimum \( k \) value which gives the maximum value for the 3\(^{rd} \) and 4\(^{th} \) parameter product
- \( k_{opt} = (t_{mc}/t_0)^{1/2} \) (\( t_{mc} \) value is usually 3-4 and retention factor (\( k \)) should be adjusted to 1.7-2.0)
- Usually the best way to adjust \( k \) is to increase or decrease surfactant concentration

Resolution in chromatography

\[ R_S = \frac{\sqrt{N}}{4} \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{k}{k + 1} \right) \]

\( f'(L, r_c) \)

\( f'(\beta, r_c, dr) \)

Stationary Phase

Resolution Optimization

- No more resolution than required.
- Depends on complexity of your sample
5.2.2019

mass distribution ratio (in MEKC micellar electrokinetic chromatography), $k_{\text{MEKC}}$

Defined as:

$$k_{\text{MEKC}} = \frac{n_{\text{mc}}}{n_{\text{aq}}} = K \frac{V_{\text{mc}}}{V_{\text{aq}}}$$

where $n_{\text{mc}}$ and $n_{\text{aq}}$ are the chemical amounts of the analyte in the micellar and aqueous phases, respectively, $K$ is the distribution constant and $V_{\text{mc}}$ and $V_{\text{aq}}$ are the corresponding volumes of the phases.

Notes:
1. In the case of an electrically neutral analyte, $k_{\text{MEKC}}$ can be calculated directly from the migration times.
2. $k_{\text{MEKC}}$ should not be confused with the retention factor in column chromatography $k$. However, $k_{\text{MEKC}}$ is analogous to the mass distribution ratio in chromatography.

$$k_{\text{MEKC}} = \frac{t_{\text{m}} - t_{\text{w}}}{t_{\text{w}} (1 - t_{\text{m}}/t_{\text{w}})}$$
Separation must take place in Migration Window (migration area) = between the detection of electroosmosis and the micelle

**MEKC - Resolution**

Factors affecting resolution in MEKC through \( N, \alpha, k, K, \beta \) and \( t_0 / t_{MC} \)

- \( \text{pH} \)
- \( \text{buffer type, concentration, strength} \)
- \( \text{micelles type, concentration} \)
- \( \text{organic modifier short or long chain} \)
- \( \text{wall coating} \)
- \( \text{temperature} \)
- \( \text{applied voltage} \)
MEKC – Method development

- start with SDS in the range 30 to 100 mM
- pH > 8, borate or phosphate buffers
- applied voltage 15 to 30 kV
- temperature controlled
- add short chain alcohols (methanol) to increase solubility in the aqueous phase
- add long chain alcohols (1-butanol) to increase solubility in the micellar phase
- ...