Micellar electrokinetic chromatography. Theory and application.

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Plan
• Principal of separation in MEKC;
• Factors influencing peaks resolution in MEKC;
• Sample preconcentration methods;
• Detectors for MEKC;
• Application of MEKC;
• Summary
Micellar electrokinetic chromatography (MEKC) – both chromatographic and electrophoretic technique.

Firstly introduced by Terabe in 1984.

Can be conducted on CE equipment with prior addition of ionic surfactant to BGS.

Partition between background solution (BGS) and ionic micelles (pseudostationary phase)

![Micelle structure](image)

Fig. 5.10. Simplified schematic representation of an ionic micelle with its most important regions

Fig.1. Micelle structure (2).
Fig. 2. Separation of analytes (PAHs) with MEKC (3).

Fig. 3. Hypothetical MEKC chromatogram for a mixture of water, analyte partitioned in micelles, and micelles (3).
Fig. 4. MEKC aromatic compounds separation with SDS and UV detection at 214 nm.

<table>
<thead>
<tr>
<th>Solute</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>benzyl alcohol</td>
<td>3</td>
</tr>
<tr>
<td>benzene</td>
<td>4</td>
</tr>
<tr>
<td>nitrobenzene</td>
<td>5</td>
</tr>
<tr>
<td>toluene</td>
<td>6</td>
</tr>
</tbody>
</table>

**Factors influencing peaks resolution in MEKC**

\[
R_S = \frac{\sqrt{N}}{4} \left( \frac{\alpha - 1}{\alpha} \right) \left( \frac{k_2}{1 + k_2} \right) \left( \frac{1 - t_0 / t_{mc}}{1 + (t_0 / t_{mc})k_1} \right)
\]

- **Plate number (N)**
  \[ N = \frac{(\mu + \mu_{co})V}{2D} \]
  - applied voltage
  - analyte's diffusion
  - composition of BGS
  - organic modifiers
  - nature of surfactant
  \( N \times 10^5 \) to 1000000 (opt. for LC: several thousands)

- **Selectivity factor (\( \alpha \))**
  \[ \alpha = \frac{k_2}{k_1} \]
  - cetyltrimethylammonium chloride
  \( \alpha = 1.02 \) (opt. for LC: \( \alpha \geq 1.2 \))

**Surfactants**

<table>
<thead>
<tr>
<th>Rite salt</th>
<th>R_1</th>
<th>R_2</th>
<th>R_3</th>
<th>R_4</th>
</tr>
</thead>
<tbody>
<tr>
<td>sodium oleate</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
<td>Na</td>
</tr>
<tr>
<td>sodium hexadecylsulphonate</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>Na</td>
</tr>
<tr>
<td>sodium tetradecylsulphonate</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>Na</td>
</tr>
<tr>
<td>sodium dehydrocholate</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>Na</td>
</tr>
</tbody>
</table>
Retention factor (k) \[ k = K \tilde{u}(C_s - C_{MC}) \]
K-partition coefficient;
\( \tilde{u} \)-surfactant molar volume in L\( \cdot \)mol\(^{-1} \);
\( C_s \)-molar concentration of surfactant.

Surfactant concentration \( k_{opt} = \sqrt{t_{mc}/r_0} \)
(opt. for LC: 1-10)

Migration time window
a length of running solution where interaction between analyte and micelles takes place
(between \( t_s \) and \( t_{mc} \))

 EOF is moderately reduced
(opt. for LC: the longer column the better resolution)

Online sample preconcentration methods

Field-enhanced sample stacking (×100—300 times)
Sample solution: surfactant added
low conductivity
pressure or electrokinetic injection

BGS: high electroconductivity
EOF is suppressed

Fig. 5. The principal of field-enhanced sample stacking preconcentration technique (8).
Sweeping (×1000-5000 times)

Sample solution:  
- free of surfactant
- the same conductivity as BGS
- hydrodinamical injection of large volume  
(e.g. to fill 90% of column volume)

BGS:  
in this example EOF is neutralised

Fig. 6. The principal of sweeping preconcentration technique (10).

• Detectors for MEKC

• Photometric detection (LoD is over 10^{-3} M)

Fig. 7. UV detector for liquid chromatography (would be similar for MEKC)

http://www.chromatography-online.org/; pp 97-98, 101.; (assessed on 30 November 2010).
Laser induced fluorescence (LIF) detection (LoD is below $10^{-9}$ M)

Fig. 8. MEKC-LIF diagram


Electrochemical detection


Fig. 9. Single fibre electrode for coupling with capillary columns (amperomitic detection)
• **Mass spectrometric detection**
  
  - High resolution power of MEKC
  
  + Excellent selectivity of MS detectors (e.g. ESI)

Problems can be eliminated with:

• BGS which contain less than 0.1% of non-volatile compounds;

• volatile surfactants:
  
  ![Ammonium Perfluorooctanoate (APFOA)](image)

  *ammonium perfluorooctanoate (APFOA)*

• polymeric surfactants

  **e.g.** *butyl acrylate/butyl methacrylate/methacrylic acid copolymer*

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• **Application of MEKC**

  - pharmaceutical analysis;
  - medical analysis;
  - food analysis;
  - environmental analysis.

  **Pharmaceutical analysis**
  
  - versatility **including** enantiomers separation;
  - high resolution;
  - fast separation;
  - MEKC procedures can be developed and validated in short period of time

MEKC is a working method of **European and U.S. Pharmacopoeia**

The objects of drug analysis:

- purity
- presence of by-products
- drug stability.
Medical analysis
- direct injection of body fluids;
- proteins are solubilised in micelles.

Environmental analysis
-separation of large number of analytes for short time

Determination of nonylphenol and nonylphenol ethoxylates in wastewater using MEKC (12)

Fig. 7. The structure of nonylphenol isomers and nonylphenol ethoxylates (12).
Fig. 8. MEKC separation of nonylphenol and oligomers of nonylphenol ethoxylates (12).

\[ \text{N=} 40-60\,000\, \text{plates} \]
\[ \text{R=} 2.5-3.5 \]
\[ \text{LoD for procedure with LLE} \quad \text{— 4-9 ppb (UV-detection at 200 nm)} \]

**Food analysis**
- agrochemicals and other food pollutants;
- food nutrients (usually at not very low content but at high number of them).

Differentiation of green tea samples by chiral CD-MEKC analysis of catechins content (13)

![Chemical structures of catechins in green tea](image)

<table>
<thead>
<tr>
<th>native catechin</th>
<th>Heat, storage</th>
<th>non-native catechin</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-Epigallocatechin</td>
<td>Heat, storage</td>
<td>(-)-Gallocatechin</td>
</tr>
<tr>
<td>(-)-Epicatechin</td>
<td>Heat, storage</td>
<td>(-)-Gallocatechin</td>
</tr>
</tbody>
</table>

Fig. 9. Native and non-native catechins in green tea
Fig. 10. 2-Hydroxypropyl-β-cyclodextrin as a chiral selector.

Optimised conditions:
• borate-phosphate buffer of pH 2;
• 90 mM of SDS with 25 mM of HP-β-CD;
• 15 kV voltage;
• temperature column 25 °C;
• UV detection at 200 nm.

Fig. 11. Green tea catechins separation with CD-MEKC (mg/g level in tea) (13).

**Summary**
MEKC separation—partition of analyte between BGS and moving ionic micelles.

The resolution depends on:

**Efficiency**—applied voltage.

**Selectivity factor**—type of surfactant, organic modifier and other additives e.g. chiral selectors, buffer concentration, pH of BGS, temperature.

**Retention factor**—surfactant concentration.

**Migration time window**—EOF.

*Chromatographic performances of MEKC usually much better than those for HPLC, which allows to complete separation of complex mixtures in a short time.*

**To increase sensitivity:**
• on-line preconcentration techniques;
• the use of laser induced fluorescence detection;
• the use of MSD like ESI.
MEKC–analysis of small organic molecules (up to peptides)

Advantages:
• high resolution for short time;
• wide capabilities of optical isomers separation;
• organic solvents free or their low consumption;
• equipment is cheaper than HPLC systems.

However, in some cases MEKC may suffer from:
• adverse effect of temperature fluctuations;
• poor reproducibility of electroosmotic flow;
• limited range of compatible detectors;
• difficulties in the use of mass detectors, particularly ESI.

References