A. Modes of separation capillary electrophoresis

1. Capillary Zone electrophoresis
2. Capillary iso-electric focusing
3. Micellar electrokinetic Capillary chromatography
4. Capillary electrochromatography
5. Capillary gel electrophoresis

B. Electrophoresis for Bio-Applications

DNA, RNA, and protein
Capillary iso-electric focusing

In this case, the electroosmotic force is weaker than electrophoretic force.
Micellar electrokinetic Capillary chromatography

Separation of neutral solute
Psuedo-stationary phase

\[ \text{OSO}_3^- \text{Na}^+ \]
Sodium dodecyl sulfate (\(n\text{-C}_{12}\text{H}_{25}\text{OSO}_3\text{Na}^+)\)

Advantage: easy to apply
Disadvantage: less selectivity

Figure 24-25 Negatively charged sodium dodecyl sulfate micelles migrate upstream against the electroosmotic flow. Neutral molecules are in dynamic equilibrium between free solution and the inside of the micelle. The more time spent in the micelle, the more the neutral molecule lags behind the electroosmotic flow.
Capillary electrochromatography

Capillary electrochromatography is an electroosmotically driven liquid chromatographic technique.

<table>
<thead>
<tr>
<th></th>
<th>Stationary phase</th>
<th>Mobile phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary electrochromatography:</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Capillary electrophoresis:</td>
<td>no</td>
<td>yes</td>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>Charged solutes</th>
<th>Neutral solutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capillary electrochromatography:</td>
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<td>yes</td>
</tr>
<tr>
<td>Capillary electrophoresis:</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>
Capillary Electrophoresis: analysis of polycyclic aromatic hydrocarbons

Stationary phase: 90% 3-μm octyldecyl-silica particles; 10% 1-μm silica

Partition stationary phase

Stabilization of Electroosmotic flow

Mobile phase: mixture of acetonitrile and 4mM sodium tetraborate solution

Capillary Electrochromatography: analysis of polycyclic aromatic hydrocarbons

Figure 2. Electrochromatogram showing the capillary electrochromatographic separation of the 16 PAHs. The column dimensions were 75 μm i.d. x 365 μm o.d. (33-cm packed length). The mobile phase consisted of 80% acetonitrile in a 4 mM sodium borate solution. The applied voltage was 15 kV. Injection was performed electrokinetically at 5 kV for 5 s. The peaks are identified as follows (N-10^-6-10^-8 M of each compound): (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benz[a]anthracene, (10) chrysene, (11) benzo[b]fluoranthene, (12) benzo[k]fluoranthene, (13) benzo[a]pyrene, (14) dibenz[a,h]anthracene, (15) benzo[ghi]perylene, and (16) indeno[1,2,3-cd]pyrene.

\[ N = 5.44 \left( \frac{t_R}{w_h} \right)^2 = 16 \left( \frac{t_R}{w_b} \right)^2 \]

Table 2. Comparison of Efficiencies between CEC and Micro-HPLC

<table>
<thead>
<tr>
<th>PAH</th>
<th>no. of theoretical plates (N)/m</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CEC</td>
</tr>
<tr>
<td>naphthalene</td>
<td>102 000</td>
</tr>
<tr>
<td>fluoranthene</td>
<td>132 000</td>
</tr>
<tr>
<td>benz[a]anthracene</td>
<td>137 000</td>
</tr>
<tr>
<td>benzo[k]fluoranthene</td>
<td>138 000</td>
</tr>
</tbody>
</table>

5. Capillary gel electrophoresis

a. Blocking the solute diffusion caused by Joule heating
b. Size of the channels in the gel gives further selection (entropy effect)

B. Electrophoresis for Bio-Applications

Separation of DNA, RNA, and protein

Electroosmosis can play a significant role in capillary gel electrophoretic separation, but not in gel electrophoretic separation. Both techniques separate solutes by their electrophoretic mobility.
Separation of DNA and RNA

Figure 2. A schematic log-log plot of reduced mobility, $\mu/\mu_0$, vs. molecular size $L$. The various regimes are discussed in Section 2. From [23], with permission.
Separation of Protein

Size effect and electrophoretic mobility
Further applications
-- an example: DNA sequencing

Polymerase Chain Reaction (PCR)
DNA Polymerase reads the template strand and synthesizes a new second strand to match:

5' - TACGCGTGATATGCTAGCTGATGAT
3' - ATGCGCGATGATGCTAGCTGATGAT

If 5% of the T nucleotides are actually dideoxy T, then each strand will terminate when it gets a ddT on its growing end:

5' - TACGCGTGATATGCTAGCTGATGAT•
5' - TACGCGTGATATGCTAGCTGATGAT•
5' - TACGCGTGATATGCTAGCTGATGAT•
5' - TACGCGTGATATGCTAGCTGATGAT•
5' - TACGCGTGATATGCTAGCTGATGAT•
5' - TACGCGTGATATGCTAGCTGATGAT•
5' - TACGCGTGATATGCTAGCTGATGAT•
5' - TACGCGTGATATGCTAGCTGATGAT•
5' - TACGCGTGATATGCTAGCTGATGAT•
5' - TACGCGTGATATGCTAGCTGATGAT•
5' - TACGCGTGATATGCTAGCTGATGAT•

One-mer difference

Electrophoresis
Automatic DNA Sequencing

One-lane: capillary electrophoresis

G, T, G, and C terminators are labeled by four different dyes respectively.
A. Important Concepts:

*Electrophoresis*

*Electrophoretic mobility* → Separation

*Capillary electrophoresis*

*Electroosmosis and Electroosmotic flow* → Driving force

*Apparent Mobility*

*Separation Efficiency*

B. Modes of separation capillary electrophoresis

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2. Capillary iso-electric focusing
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C. Bio-Applications
Separation Sciences

1. Introduction: Fundamentals of Distribution Equilibrium

2. Gas Chromatography (Chapter 2 & 3)

3. Liquid Chromatography (Chapter 4 & 5)

4. Other Analytical Separations (Chapter 6-8)
   a. Planar chromatography
   b. Supercritical fluid chromatography
   c. Electrophoresis
   d. Centrifugation
   e. Field Flow Fractionation
Homework III (b)

1. What is electrophoresis and electroosmosis?

2. Explain how neutral molecules can be separated by micellar Electrokinetic capillary chromatography.

3. Compare HPLC and Capillary electrochromatography.

4. Compare capillary gel electrophoresis and gel electrophoresis.

5. The observed behavior of benzyl alcohol (C₆H₅CH₂OH) in capillary electrophoresis is given below. Explain what happens as voltage is increased.

<table>
<thead>
<tr>
<th>Electric field (V/m)</th>
<th>Number of plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>6400</td>
<td>38000</td>
</tr>
<tr>
<td>12700</td>
<td>78000</td>
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<tr>
<td>19000</td>
<td>96000</td>
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<td>31700</td>
<td>124000</td>
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<tr>
<td>38000</td>
<td>96000</td>
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</table>