Agilent CE System

A Quick Start Guide to Maintenance and Troubleshooting
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Practical Considerations

Capillary
- One capillary per method
- Re-cut end if broken or replace
- Keep window clean (and optical interface)
- Storage: flush with water and air
- When in doubt, throw it out

Sample
- Remove particulates (filter, centrifuge)
- Thermostat carousel if temperature sensitive or small volume

Buffers
- Do not overuse (e.g. replenish often)
  - overuse can cause migration time drift, spurious peaks
- Try to use buffers not just electrolytes
  - electrolysis can change the pH and cause migration time drift
- Use highest quality buffer components available
- Remove particulates with 0.2 or 0.45 µm filter
- Use CE quality water (18 MΩ and organics removed)
- Use good, calibrated pH meter (<0.1 pH units) or purchase pre-made buffers
- Prepare fresh buffer often
  - phosphate and organic buffers susceptible to microbial growth
Practical Considerations

Data Analysis

- Reproducibility
  - Migration time %RSD typically 0.3–1%
  - Area %RSD typically 1–3%

- Peak shapes
  - Non-Gaussian peak shapes may be electrophoretic phenomena and are not indicative of poor separations
  - Tailing peaks (particularly of proteins and quaternary amines) can be improved through buffer optimization or coated capillary (e.g. PVA)

- Quantitative analysis
  - Use peak area, not height (height is dependent on stacking)
  - Use corrected area (A/t) routinely, especially for chiral analysis and when migration time shifts
  - External (or internal) standard calibration required
  - Check integration carefully
  - Acquire sufficient number of data points—at least 15–20 over the peak
## Practical Considerations

### Factors Affecting Reproducibility of Mobility and Migration Time

<table>
<thead>
<tr>
<th>Factor</th>
<th>Cause/effect</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature change</td>
<td>Changes viscosity and EOF</td>
<td>Thermostat capillary</td>
</tr>
<tr>
<td>Adsorption to capillary walls</td>
<td>Changes EOF (caused by buffer, additive, or sample adsorption)</td>
<td>Condition capillary and allow sufficient equilibration time</td>
</tr>
<tr>
<td>Hysteresis of wall charge</td>
<td>Caused by conditioning capillary at high (or low) pH and employing a low (or high) pH running buffer</td>
<td>Avoid pH differences Allow sufficient equilibration time</td>
</tr>
</tbody>
</table>
| Changes in buffer composition | pH changes due to electrolysis  
Buffer evaporation  
Conditioning waste flushed into outlet vial  
Carrying sodium hydroxide from conditioning vial into buffer vial, for example | Replenish buffer  
Cap buffer vials and cool carousel  
Use separate vial to collect wash solutions  
First dip capillary in separate buffer or water vial |
| Buffer reservoirs not level | Non-reproducible laminar flow  
If not replenishing, do not use inlet vial for washing capillary | Level liquid in vials |
| Different silanol content of silica batches | Different wall charge and variations in EOF | Measure EOF and normalize, if necessary |
| Variations in applied voltage | Proportional changes in migration time | Not user accessible |
### Factors Affecting Peak Area Reproducibility

<table>
<thead>
<tr>
<th>Factor</th>
<th>Cause/effect</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature change</td>
<td>Changes viscosity and EOF</td>
<td>Thermostat capillary</td>
</tr>
<tr>
<td>Sample evaporation</td>
<td>Increasing sample concentration</td>
<td>Cap vials and/or cool carousel</td>
</tr>
<tr>
<td>Instrumental limitations</td>
<td>System rise time significant proportion of injection time</td>
<td>Increase injection time</td>
</tr>
<tr>
<td>Sample carry-over</td>
<td>Extraneous injection</td>
<td>Use capillary with flat smooth injection end</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Remove polyimide from end of capillary</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dip capillary in buffer or water after injection</td>
</tr>
<tr>
<td>Zero-injection caused by simply dipping the capillary in the sample</td>
<td>Extraneous injection</td>
<td>Cannot be eliminated but can be quantified</td>
</tr>
<tr>
<td>Sample adsorption to capillary walls</td>
<td>Distorted peak shape</td>
<td>Change buffer pH</td>
</tr>
<tr>
<td></td>
<td>Non-eluting sample</td>
<td>Increase buffer concentration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Use additive or coated capillary</td>
</tr>
<tr>
<td>Low signal-to-noise ratio</td>
<td>Integration errors</td>
<td>Optimize integration parameters</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increase sample concentration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Use peak height</td>
</tr>
<tr>
<td>Sudden application of high voltage</td>
<td>Heating, thermal expansion of buffer, and expulsion of sample</td>
<td>Ramp separation voltage</td>
</tr>
<tr>
<td>Electrokinetic injection</td>
<td>Variations in sample matrix</td>
<td>Use hydrodynamic injection</td>
</tr>
</tbody>
</table>
Preventive Maintenance

Injection System

Electrodes
- Do not open top cover or remove cartridge without lowering lifts
  - Electrodes can fill with buffer leading to carryover
- Clean frequently (e.g. weekly)
  - Remove, rinse with water then isopropanol, air dry or use compressed air
- Inspect for
  - Salt deposits
  - O-ring integrity
  - Bending

Prepunchers
- Clean frequently (e.g. weekly)
  - Remove, rinse with water then isopropanol, ultrasonicate, air dry or use compressed air
- Inspect
  - Salt deposits
  - Bent tip
  - Deposits in top funnel

Replace if either electrodes or prepunchers are bent or cannot be cleaned
Preventive Maintenance

Replenishment System

System cleaning
- Flush tubes or use multiple replenishment cycles
- Perform when new buffer is added to reservoir
- Clean with isopropanol/water
- Filter (0.2 or 0.45 µm) all solutions prior to use

Replenishment needle
- Keep water-filled vial in carousel position 49
- Inspect for bending
- If clogged, remove and syringe flush with water/isopropanol

O-ring in buffer reservoir cap
- Inspect for damage
- Remove salt deposits

Inlet frit
- Inspect and clean (backflush)
- Replace when dirty

Vial Caps
- Use polyurethane caps for resealing (5181-1512)
- Inspect for damage

Do not use buffer reservoir for long-term storage (prevents oxygen saturation)
- Use insert for overnight runs
- Use argon supply for pump inlet
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**Preventive Maintenance**

**Detection System**

**Optical alignment interface**
- Inspect for occlusion under microscope or magnifier
  - Dust or shards of polyimide
- If capillary is broken while in the interface, inspect and remove fragments
- Clean with air burst or isopropanol/water

**Lamp**
- Perform DAD test regularly (e.g. weekly) and monitor lamp counts
  - Use alignment interface without capillary to monitor lamp counts
- Low lamp counts or noisy baseline can be caused by
  - Old lamp (>700 hours)
  - Occluded alignment interface

**Capillary window**
- Gently clean with isopropanol and lint-free wipe

**DAD Test**

![DAD Test Graphs]
# Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstable current</td>
<td>Capillary not filled with buffer</td>
<td>Increase flush time</td>
</tr>
<tr>
<td></td>
<td>Air bubbles in buffer</td>
<td>Ultrasonicate buffer</td>
</tr>
<tr>
<td></td>
<td>Capillary clogged</td>
<td>Remove via flush with syringe (offline)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Replace capillary</td>
</tr>
<tr>
<td>Poor resolution or broad peaks</td>
<td>Capillary not conditioned</td>
<td>Flush and repeat analysis</td>
</tr>
<tr>
<td></td>
<td>Sample overloaded</td>
<td>Reduce sample concentration or amount injected</td>
</tr>
<tr>
<td>No signal</td>
<td>Wrong setting of power supply polarity</td>
<td>Verify injection and migration is toward anode</td>
</tr>
<tr>
<td></td>
<td>Detection wavelength incorrect</td>
<td>Verify solute absorption and signal wavelength</td>
</tr>
<tr>
<td></td>
<td>Sample not injected</td>
<td>Verify no air bubble trapped in bottom of sample vial</td>
</tr>
<tr>
<td>Noisy baseline</td>
<td>Buffer contains particulates</td>
<td>Filter through 0.2 or 0.45 µm filter</td>
</tr>
<tr>
<td></td>
<td>Alignment interface occluded</td>
<td>Examine under microscope and clean with water/isopropanol</td>
</tr>
<tr>
<td></td>
<td>Use reference wavelength</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Capillary window dirty</td>
<td>Examine and clean with lint-free paper/MeOH or isopropanol</td>
</tr>
<tr>
<td></td>
<td>Lamp is old</td>
<td>Replace if more than 650–750 hours</td>
</tr>
<tr>
<td>Poor reproducibility</td>
<td>Capillary not equilibrated</td>
<td>Increase flush time with buffer</td>
</tr>
<tr>
<td></td>
<td>Buffer overused</td>
<td>Replace buffer</td>
</tr>
</tbody>
</table>
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**CE Method Development**

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**Perform CZE experiment using following buffers:**
- 25 mM phosphate (pH 2.5)
- 25 mM borate (pH 9.3)
- 25 mM borate, 25 mM sodium dodecyl sulfate (pH 9.3)

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**Sample**
- dissolved in water, diluted buffer or other minimally conductive matrix

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**Do you know the sample's ionic character (pKa)?**

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**Determine the analyte's charge**

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**Perform CZE experiment**
- Cationic: Initial run 25 mM phosphate (pH 2.5)
- Anionic: Initial run 25 mM borate (pH 9.3)
- Neutral: Initial run 25 mM borate – 25 mM sodium dodecyl sulfate (pH 9.3)

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**Check concentration by flow-injection analysis**

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**Any peaks?**
- YES
- NO

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**If sample is too dilute:**
1. Use high sensitivity cell
2. Concentrate sample
3. Use electrokinetic injection

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**If sample contains high salt concentration:**
- Desalt or increase the buffer concentration

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* More details can be found on the CE-Partner CD-ROM (publication number 5968-3234E)
CE Method Optimization

Optimize separation for MEKC conditions

Correct number of peaks?
Is peak shape good?
Is resolution sufficient?

YES

NO

Increase sodium dodecyl sulfate concentration from 25 mM to 100 mM in 25 mM increments and/or decrease pH to 7 or 8

Correct number of peaks?
Is peak shape good?
Is resolution sufficient?

YES

NO

Optimize separation for CZE conditions

Correct number of peaks?
Is peak shape good?
Is resolution sufficient?

YES

NO

Are the separation time and selectivity optional?

YES

NO

Adjust variables (in the following order):
1. Optimize the pH
2. Increase the buffer concentration in 25 mM increments
3. Change the buffer ion
4. If peaks are tailing, add a buffer modifier such as hydroxymethylcellulose or use a coated capillary

Optimize
1. Injection
2. Capillary length
3. Capillary inner diameter
4. Capillary voltage
5. Capillary temperature

Determine the analyte's charge

Does the method meet the analysis criteria?

YES

NO (MEKC)

NO (CZE)

Method completed