Agilent CE System

A Quick Start Guide to Maintenance and Troubleshooting

Agilent Technologies

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

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

Factors Affecting Reproducibility of Mobility and Migration Time

Factor	Cause/effect	Solution
Temperature change	Changes viscosity and EOF	Thermostat capillary
Adsorption to capillary walls	Changes EOF (caused by buffer, additive, or sample adsorption)	Condition capillary and allow sufficient equilibration time
Hysteresis of wall charge	Caused by conditioning capillary at high (or low) pH and employing a low (or high) pH running buffer	Avoid pH differences Allow sufficient equilibration time
Changes in buffer composition	pH changes due to electrolysis	Replenish buffer
	Buffer evaporation	Cap buffer vials and cool carousel
	Conditioning waste flushed into outlet vial	Use separate vial to collect wash solutions
	Carrying sodium hydroxide from conditioning vial into buffer vial, for example	First dip capillary in separate buffer or water vial
Buffer reservoirs not level	Non-reproducible laminar flow	Level liquid in vials If not replenishing, do not use inlet vial for washing capillary
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

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

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

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

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

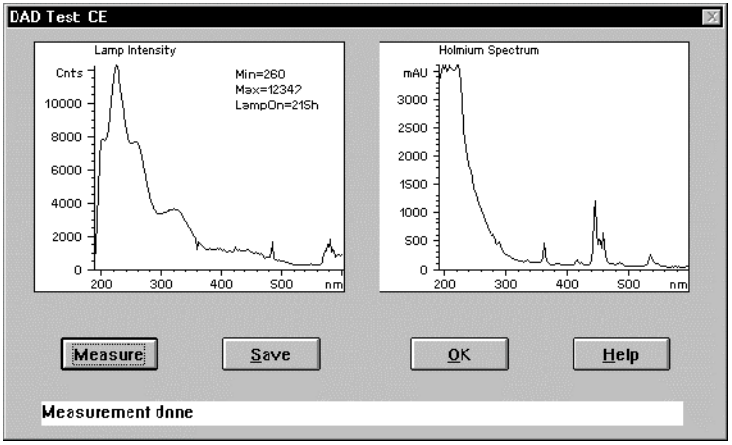
Lamp

- 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

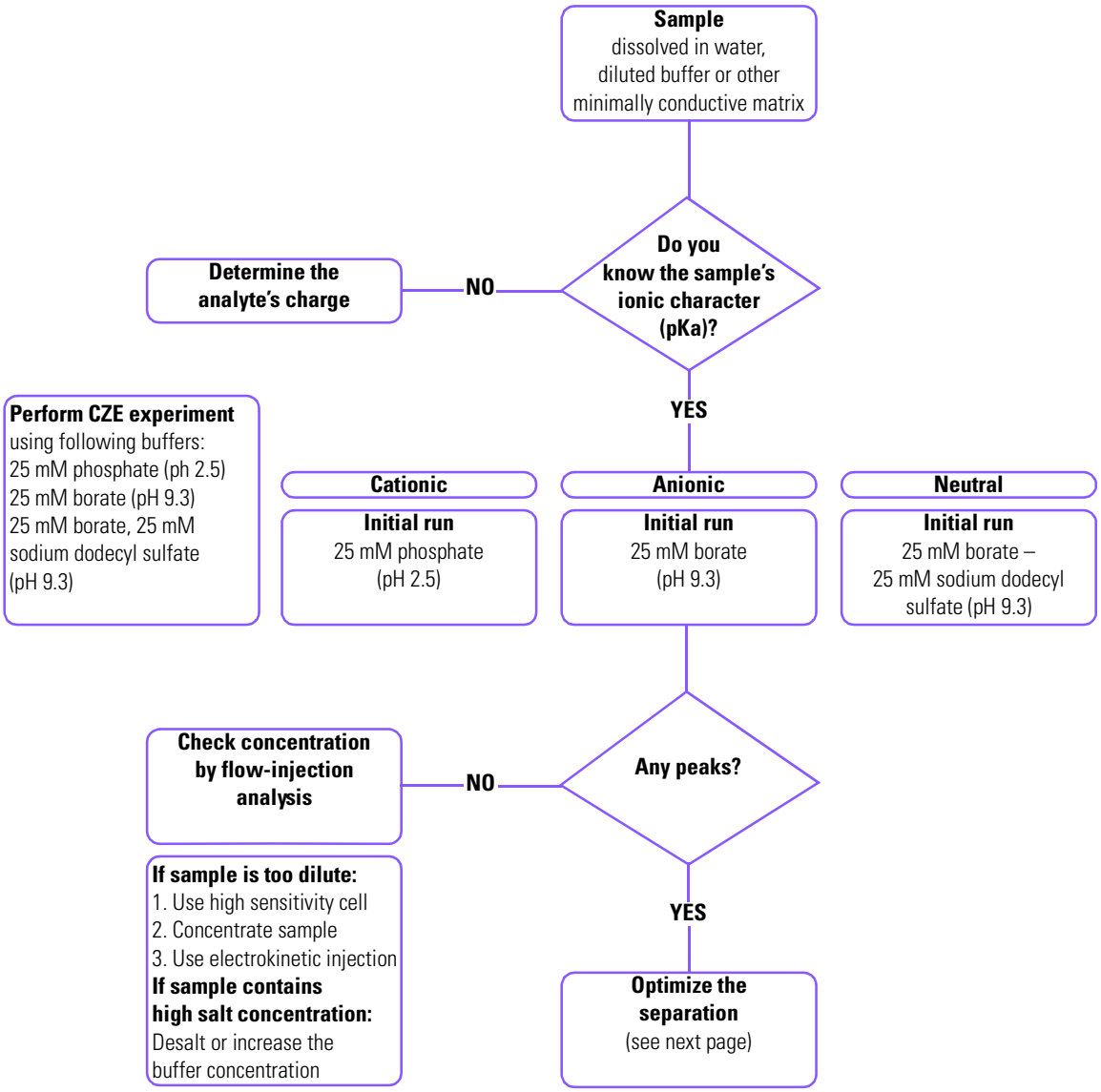
DAD Test



Troubleshooting

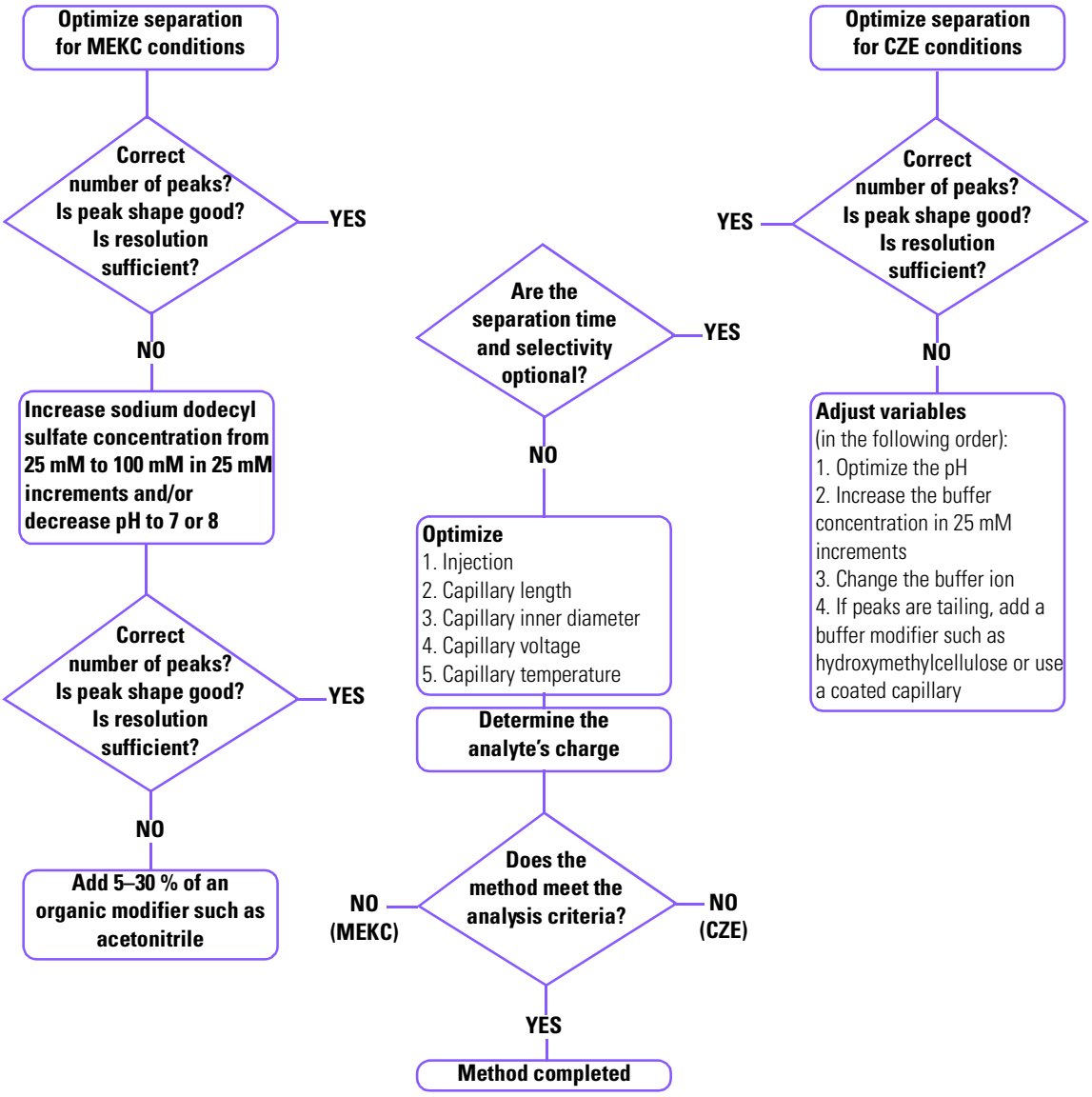
Problem	Possible Cause	Solution
Unstable current	Capillary not filled with buffer	Increase flush time
	Air bubbles in buffer	Ultrasonicate buffer
	Capillary clogged	Remove via flush with syringe (offline)
		Replace capillary
Poor resolution or broad peaks	Capillary not conditioned	Flush and repeat analysis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

CE Method Development*



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

CE Method Optimization



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