Standard Operational Procedure for Capillary Electrophoresis

*Please ask an experienced operator for training before you use the CE if you can. A demo is most probably more efficient than a SOP, considering the nature of our CE operation.

What You Will Do

To prepare the CE instrument for the analysis you need to do the following tasks:

- start the Agilent CE instrument,
- start the computer,
- start the Agilent ChemStation software,
- allow the lamp time to warm up (approximately 1 hour),
- prepare vials for sample and buffer,
- insert a capillary into the alignment interface,
- insert the capillary into the capillary cassette,
- insert the capillary cassette into the system, and
- prepare the Replenishment system (optional).

After you have completed all these tasks you can create a CE method.

1. Solution Preparation

Multiple solutions (aqueous buffers, background electrolytes, flushing solutions) may be required in method development stage. All of these solutions must be **filtered** (using 0.45 mm or smaller pore size filters) and **degassed** (sonication for about 10 minutes) prior to introduction to the instrument. Failure to do so can result in capillary blockage and problematic data collection. Use only recommended vials when working with the CE instrument. Make sure vials have been filled enough solution. Insert the vials into the sample tray and write down the positions of each vial for method creation later.

- solution levels of **sample vials**

The level of sample in the vials depends on the injection technique used.

Hydrodynamic injection:

When using injection by pressure the sample level only needs to touch the capillary. The minimum liquid level in the sample vials for this technique is 5 mm.

Electrokinetic injection:

When using injection by voltage or current the sample level needs to touch the electrodes. Therefore the minimum liquid level in the sample vial is 1 cm.

- solution levels of **Buffer Vials**

When the run is started the liquid level in the vial should touch the electrodes to enable a current to flow. Therefore the minimum liquid level in the buffer vials is 1 cm. A buffer level of 1.4 cm is recommended.

- solution levels of **Outlet Vials**

Outlet vials (for example, waste vials) should also be filled with enough liquid to touch the capillaries. Thus the liquid flow through the capillary does not produce droplets at the capillary end, which can cause pressure. This can influence the injection amount. If the waste vial is filled with liquid, outgoing droplets

cannot be drawn up the electrode by capillary effects but are flushed completely out of the outlet electrode. Therefore they cannot contaminate the lift head or the next outlet vial.

NOTE!!! Outlet vials that are used during analysis (for example, for fraction collection) need to be filled to a higher level (1 cm). The liquid must touch the electrode to enable a current to flow.

2. Turn on the CE

   Always power on the CE instrument first before you turn on the computer and open the Chemstation software. The computer sometimes has a hard time connecting to the instrument if you turn them on in an opposite way. After startup of the instrument the CE State shows **not initialized** in the Status window (Figure 1). Choose **System INIT** from the **instrument** menu, to initialize the system (Figure 1). During initialization the instrument tests different functions and adjustments. The tray as well as all three lift stations are moved to a defined position, and the lamp is switched on. Allow the lamp to warm up for one hour.

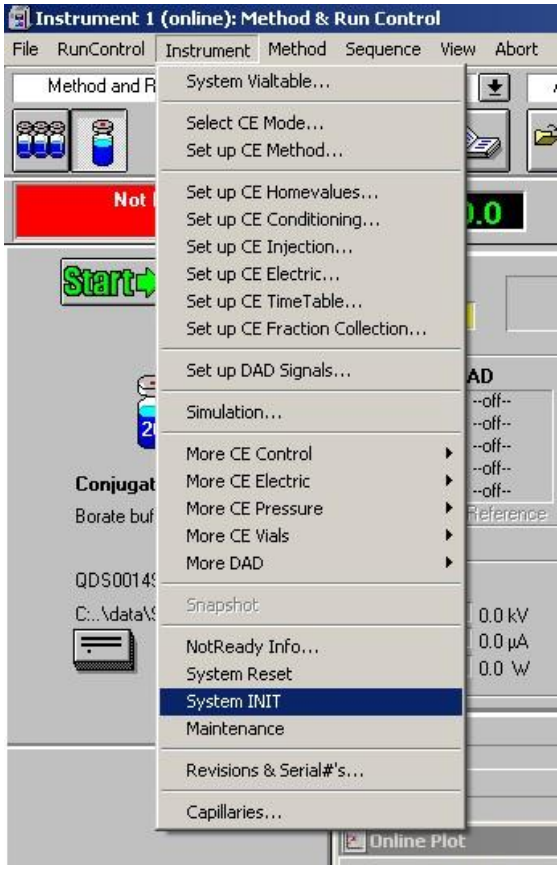


Figure 1. System Initialization

3. Cutting a New Capillary

Caution!!! Eye protection goggles and gloves must be worn when cutting and installing a capillary.
If you are using bare-fused silica capillaries which we have in the lab on spools, it will be necessary to cut a length of capillary for usage on the CE. Cutting a capillary takes practice, therefore smaller lengths (10-15cm) are recommended to use at first.

1) Measure the desired length of capillary from the spool. Mark this length on the polyimide coating with a sharpie. Cut at the mark with a ceramic cutter using a firm but even pressure at a 45 degree angle to the capillary. The scoring should be done with one distinct motion, not by sawing!
2) Next, measure and mark the detection window, which should be located about 8-9 cm from one end of the capillary.
3) Scrap the coating of detection window with the Fujikura FSM-05S fusion splicer (Figure 2 Left): lift the microscope and two clamps (Figure 2 Right), level the capillary into the capillary trap and make sure the detection window mark is in the middle, then close the two clamps and the microscope. Watch through the microscope when pressing the SPLICE button: the polyimide coating of the capillary glows red and chars (about 1 sec). The detection window should be approximately 1.5-2mm wide. Larger detection windows will actually have adverse effect on detection.

Caution!!! The scraped capillary will become super fragile. Be careful handling the capillary from now on. Avoid touching and bending the detection window area. To take the capillary off the fusion splicer, lift the microscope and then lift BOTH clamps AT THE SAME TIME to free the capillary. You may want to wet a disposable wipe with DI water and GENTLY wipe the soot from the window.

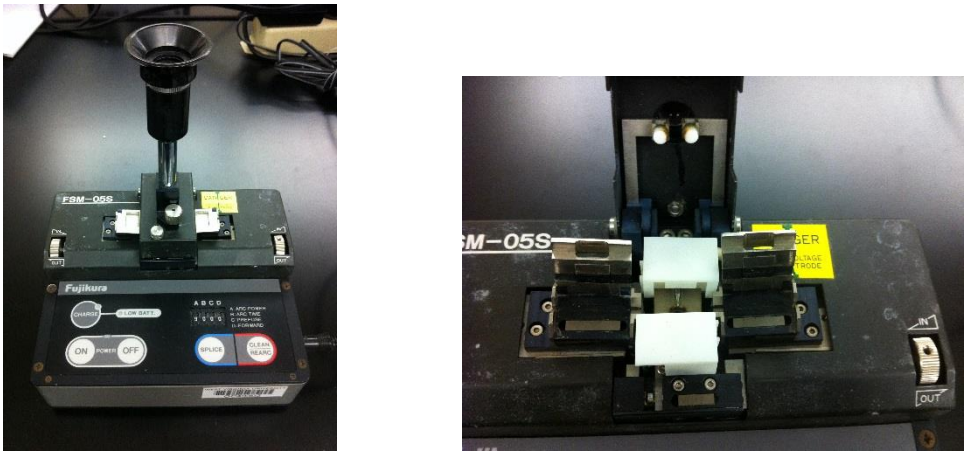


Figure 2. Fujikura FSM-05S fusion splicer

4) Insert the capillary into an alignment interface (please choose the alignment interface according to the capillary size and Agilent's color code (Table 1)). To make sure the detection window is properly placed in the middle of the alignment interface, you should use a microscope to check it.

Table 1. Alignment Interface Color Codes

Alignment Interfaces

Sleeve Color	Part Number	Used For*
Black	G1600-60150	25 µm id capillaries with extended light path
Green	G1600-60210	50 µm id straight capillaries
Red	G1600-60230	50 µm id capillaries with extended light path
Blue*	G1600-60310	75 µm id straight capillaries
Yellow	G1600-60330	75 µm id capillaries with extended light path

* For 100 or 150 µm od capillaries use the blue alignment interface

5) Now the capillary is ready to be put into the cassette.

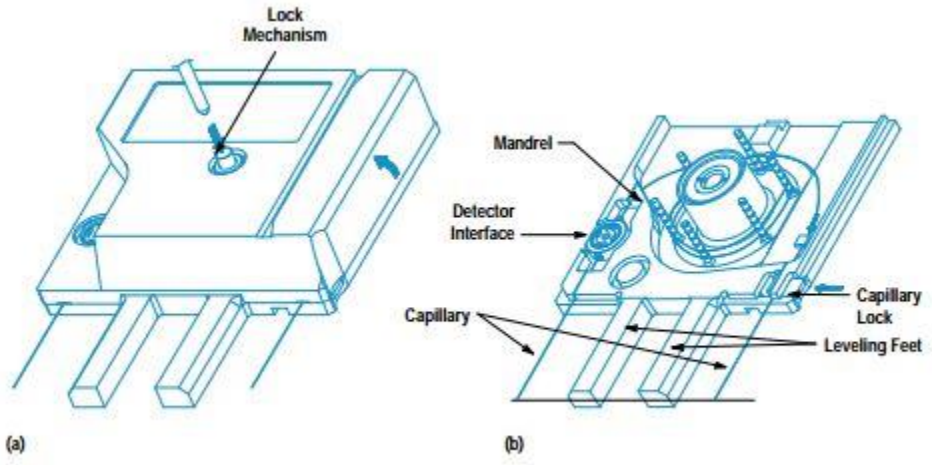


Figure 3. CE Cassette

Open the cassette: With the cassettes (Figure 3) we have in the lab right now, you can open the cassette by holding the bottom part with the two cassette guiding pins away from you, push the button on the top part to unlock it, and push the top part to the alignment interface side (to the right if you are holding it the way as above) to open it. Lift and remove the top part.

Install the capillary: Hold the capillary at the alignment interface. Insert the alignment interface into the alignment interface holder on the cassette. Make sure the flat side of the alignment interface is at the outer edge of the cassette. Make sure the capillary slides into the capillary guiding slit and a guiding hook above the area of the alignment interface. Wind the capillary around the reel in the center of the capillary cassette. The pins surrounding the reel have grooves to secure the capillary. Insert the other end of the capillary into the guiding hook on the left. Press the white capillary lock towards the center of the cassette and insert the capillary into the capillary lock. Release the capillary lock to secure the capillary. Make sure the two capillary ends outside the cassette are the same length as the cassette guiding pins and are parallel to the pins. Use a sheet of paper or another soft item with a straight line to check this. Use the capillary lock to make adjustments on the inlet side.

Close the Capillary Cassette: Place the top of the cassette onto the bottom part with the alignment side (right side) clicked in first. Close the top and make sure the cassette cover and body form one unit. Make sure the two capillary ends outside the cassette are both the same length as the cassette guiding pins and are parallel to them. Use the capillary lock to make adjustments to the inlet side of the capillary. Push down the top button to lock the whole cassette.

6) Insert the cassette into the CE

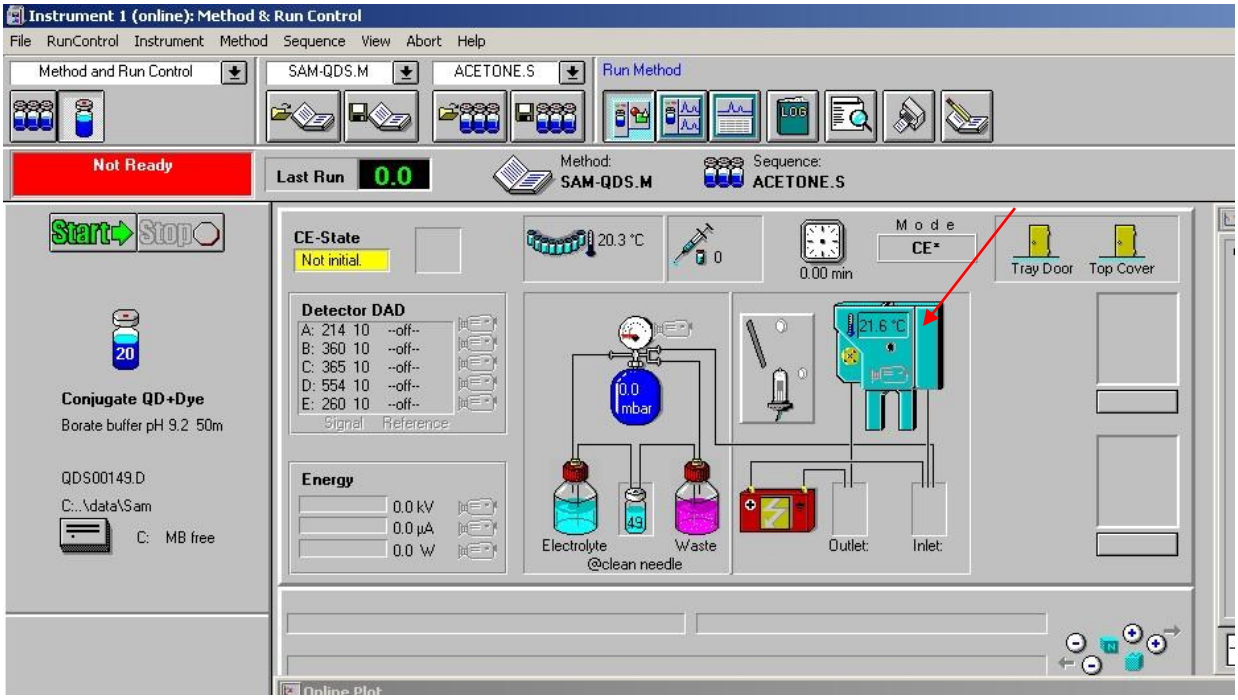


Figure 4 Cassette Image in Chemstation

Caution!!! Always right click on the cassette image in the software (Figure 2) and choose Change Cassette to relieve the two default inlet and outlet vial and access an already installed cassette, to avoid contaminations.

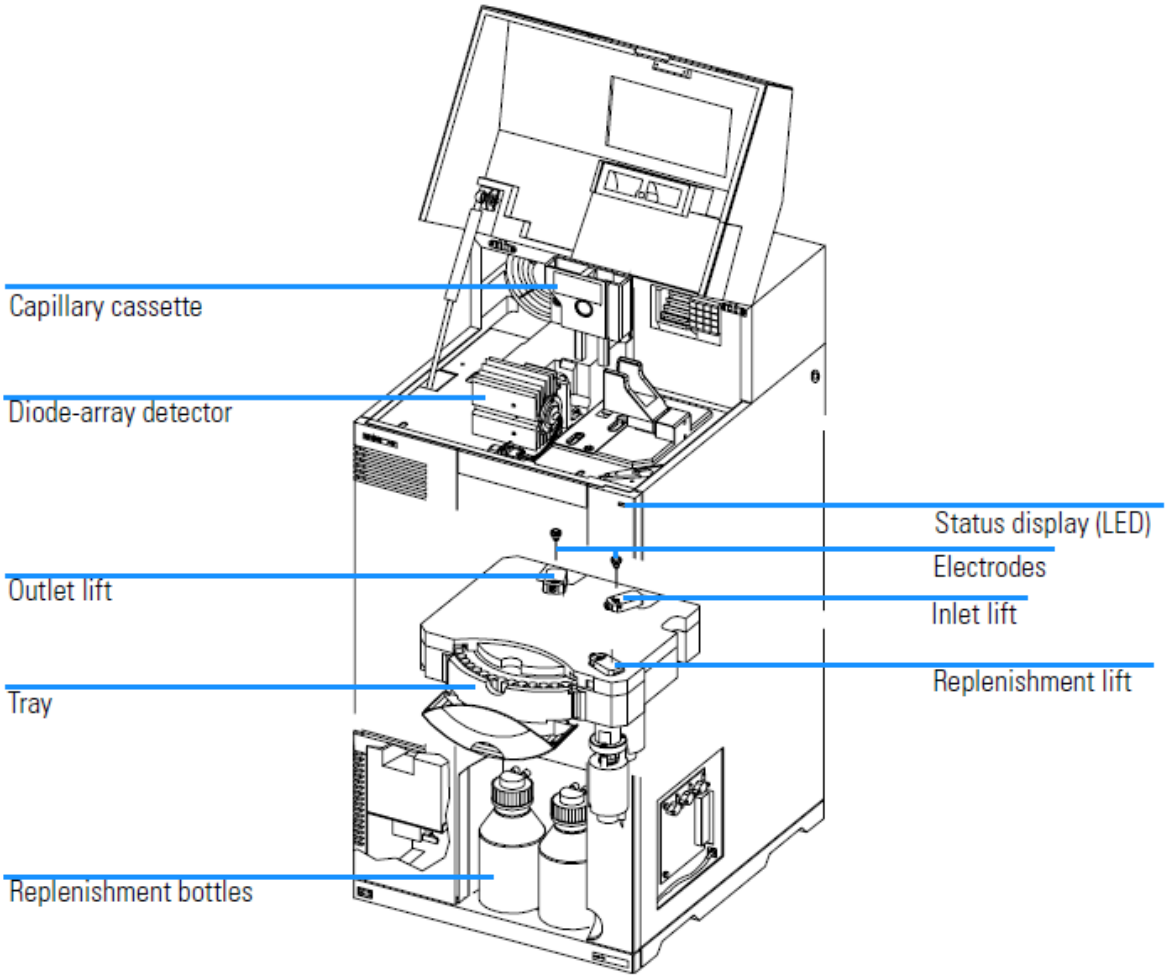


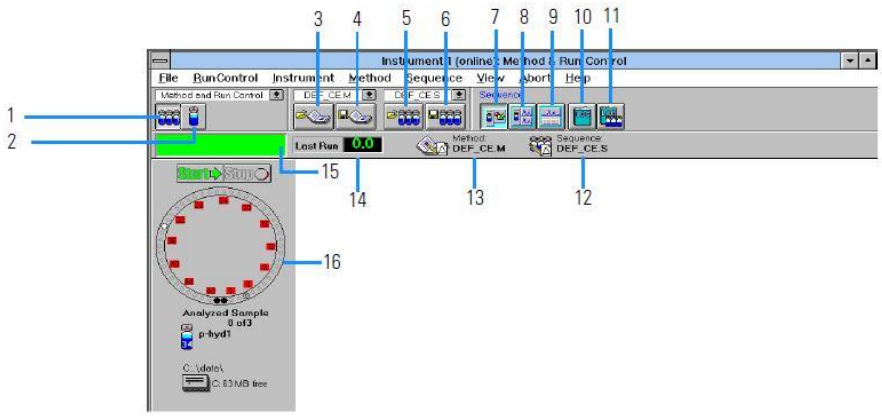
Figure 5. CE Hardware Components

Open the top cover of CE by pressing the button in the middle of the lid front to release the latch. Move the cassette guide to the front. Insert the cassette into the guiding slit of the cassette guide. Make sure the ends of the capillary are not bent. Slowly lower down the cassette and make sure both ends are into the capillary seats of the electrodes (You can guide both end of the capillary with your hand before you actually lower it all the way down).

Caution!!! Make sure your capillary ends are straight and they are into the seats before you push it all the way down. Capillaries easily break at this step.

Move the cassette guide back again once you have the cassette fully inserted. Now the alignment interface aligns the capillary automatically to the detector. Close the top cover.
The Chemstation automatically tests the wavelength calibration of the detector and advises you to do adjustments if necessary. After adjustment, you can log your capillary size and length information into the capillary information table.

4. Graphical User Interface Explanation - Chemstation

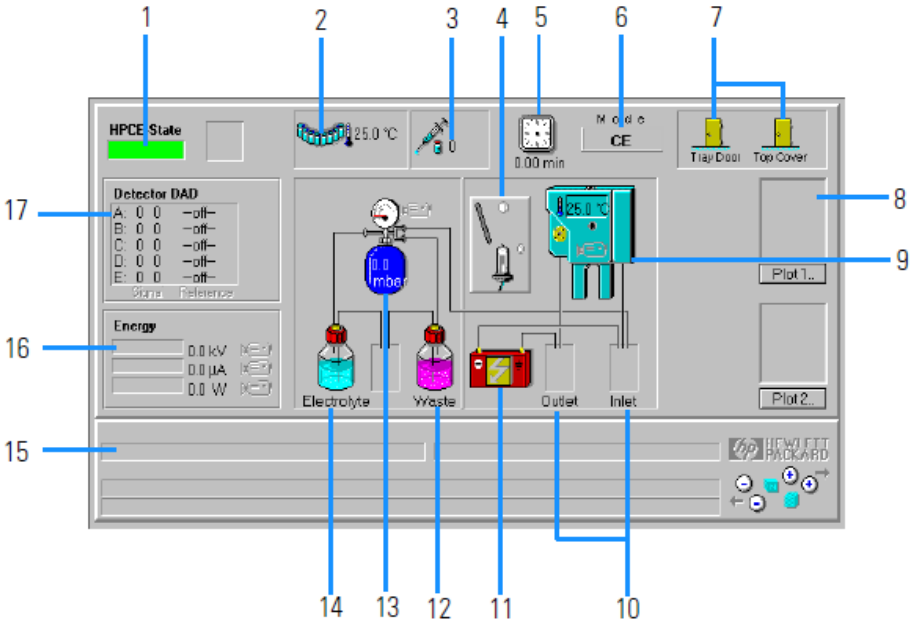


1 Work with sequences
2 Work with single vial
3 Load method
4 Store method
5 Load sequence
6 Store sequence

7 Change the layout to show the sampling area and the diagram
8 Change the layout to show the sampling area and two online samples
9 Change the layout to show the sampling area and online signal display
10 Display the current logbook
11 Display the current sequence logbook
12 View the current sequence
13 View the current method
14 View the run time
15 Status display
16 Displays sample tray when working with sequences otherwise shows single vial information

Figure 6. Common Tool Bars

Components of the Agilent CE Instrument in the CE Diagram



1 CE status
2 Tray
3 Injection
4 Diode array detector
5 Timing
6 Analyses modes
7 Doors
8 On line windows
9 Cassette temperature
10 Inlet/outlet lift
11 Electrical values
12 Waste bottle
13 Internal pressure
14 Electrolyte bottle
15 Status bar
16 Energy values
17 Detector wavelengths/spectra

Figure 7. Components of CE Instrument in CE Diagram

5. New Capillary Conditioning
   A new capillary must be conditioned prior to first use. Conditioning charges the exposed silanol groups on the inner walls of the bare fused-silica and allows for a proper electroosmotic flow. To condition a new bare silica capillary, it needs to be flushed with 1M NaOH for at least 2 hours at 20psi. An alternative flush is 0.1M NaOH overnight. After completing the flush procedure, both ends of the capillary should be stored in deionized water until the next use.

6. Create a new method
   Go to **Method** menu and choose **new method** to create a new method.
   6.1. Method Comments
   Leave here a note describing your method.
   6.2. Home values
   - Lift Offsets
     Lift offset is the distance between the bottom of the vial and the end of the capillary. Default value is 4mm, which means you should have at least 30 µl in the 200 µl vials or ….. µl in the 1 ml vials.
   - Cassette Temperature
     This temperature reflects the temperature of the cassette or the capillary part (NOT THE SAMPLE TRAY). You can lower down the temperature for the fragile bio-sample separations, though keep in mind the temperature also influences the performance of separation.
   - External Pressure
     In CEC mode the external pressure can be set which keeps the inlet AND the outlet of the capillary pressurized. This may be necessary to suppress bubble formation inside the capillary. Any value between 2 and 12 bar can be set.
   - Inlet Home vial and Outlet Home Vial
     The Inlet Home vial and Outlet Home vial define the default buffer vials that will be used for the separation (background electrolytes, BGE). **Both** inlet and outlet vials should be filled with BGE. Inlet Home refers to the vial at the capillary inlet, where the voltage is applied. Outlet Home (GND) refers to the outlet vial, at the detector end of the capillary.

   6.3. Conditioning
   - Replenishment
     Refer to a manual if you want to replenish your buffers automatically by CE. Otherwise, choose NONE replenishment.
   - Conditioning of the Capillary
     In order to have stable conditions for your analysis conditioning of the capillary is important. Depending on the separation mode used it can comprise:
     • conditioning the capillary with conditioning agents, and
     • equilibrating the capillary with buffer.
     These conditioning steps can be programmed using a table in the Preconditioning or Postconditioning section of the Conditioning screen depending on whether they should be done before or after injection and run.

Important!!! Please consider at least precondition with 1M or 0.1M NaOH, water, and your BGE buffer for each run. If you use incompatible flushing solutions in this step, such as both HCL and NaOH, please make sure these incompatible solutions always add a short flushing with water in-between to avoid contamination.

6.4. CE Injection

You have the choice to choose Hydrodynamic, Electrokinetic, Using an Injection Program or No injection here.

- Hydrodynamic

Injection by pressure is the most frequently used injection technique. There are no differences in injection concentration for molecules with different mobilities as in electrokinetic injection. In hydrodynamic injection the inlet buffer reservoir is replaced with the sample vial. A pressure is applied for a certain time to introduce the sample in the capillary.

- Electrokinetic

This injection technique is used for capillaries filled with fixed or cross-linked gels or other material of high viscosity, where injection by pressure is not applicable. In electrokinetic injection the inlet buffer vial is replaced with the sample vial. A voltage, current or power is applied for a certain time that causes the sample to migrate into the capillary.

- Difference between Hydrodynamic and Electrokinetic Injection

To do electrokinetic injection the **electrode** must touch the sample in the sample vial. Whereas in hydrodynamic injection the sample only needs to touch the **capillary tips**.

- Using an Injection Program

The injection table is used for advanced injection tasks such as: injection from different vials, multiple injection modes, e.g. spiking, and injecting a buffer plug after the sample to prevent sample loss after applying voltage. Please refer to the online help system for more information.

6.5. CE Electric

- Polarity

The standard polarity setting is positive polarity, which is the positive electrode is at the inlet vial. You can reverse the polarity to negative. This means that the inlet vial becomes the negative electrode where a negative voltage is applied. The outlet electrode is always grounded to ensure that the potential at the point of detection is close to ground.

- Voltage, Current, or Power

Voltage, current and power are related to each other by the resistance of your capillary/buffer system. The three individual values are treated as limits. The limit that is reached first applies. So you just have to set up one, such as voltage, and set the other two as syslimit.

- Lower alarm limit for current

When using constant voltage mode, the current is normally the indicator of the stability of the system. A rapid current decrease or drop can indicate an instability of the system. By setting a lower alarm limit you can define when you want to be warned of such instable conditions during the run. Instable conditions can be caused by:

no buffer in the capillary, air bubble in the capillary, clogged capillary, or broken capillary. When the lower Alarm Limit is reached during the Run part of the analysis (when the HV is applied) your current run is stopped. During a sequence the system continues with the next analysis.

Caution!!! Do not use the Lower Alarm Limit when changing the inlet or outlet vial during the run (for example for fraction collection). Otherwise the system will stop when changing the vial because the lower alarm limit is reached.

6.6. Timetable

In the CE Timetable you can choose to:

• specify the raw data that can be monitored and stored in the data file

• define stoptime and posttime of your analysis

• time-program certain events

If you are not using a constant current run and you want to run gradient of voltage or temperature, change buffer, apply pressure, change the outlet vial, change the alarm limits when changing buffers during the analysis, you can use the timetable to set values to change at a certain time during the run.

Note: time table is not execible when fraction collection has been activated.

6.7. DAD signals or detector parameters

- Signals

It is recommended to do runs without using reference wavelengths to minimize baseline noise. Reference wavelengths can help to compensate for signal drifts. As reference wavelengths are measured at higher wavelengths, the baseline noise is increased when using reference wavelengths. Therefore, it is recommended to record the two signals at the same wavelength; one using the reference wavelength and one without.

- Spectrum

To save the disk space, this function is **usually disabled**. You can define at which points on a signal, spectra will be taken and saved. You can choose to acquire them: at the upslope, apex and downslope of the peak, all in a peak, all spectra during analysis, or none. You can combine this setting with a wavelength range. By restricting the wavelength range to the range of your interest you can save some disk space; the size of your data files will be smaller because the full spectrum is not stored.

Note!!! You need to store the spectrum when you are using fraction collection function.

- Time

You can define a special stoptime and posttime for your detector, also saving some lifetime of the lamps.

- Detector Time Table

By time programming these parameters you can optimize the required disk space for your spectra by switching the spectra acquisition on and off. Thus you can acquire spectra for parts of the electropherogram only.

7. Run a sequence

You can set up consecutive runs by setting up a sequence by clicking sequence table in sequence menu (Figure 8). You can specify the sample location, method you want to use, and how many repeat runs for each sample in the sequence table (Figure 8)

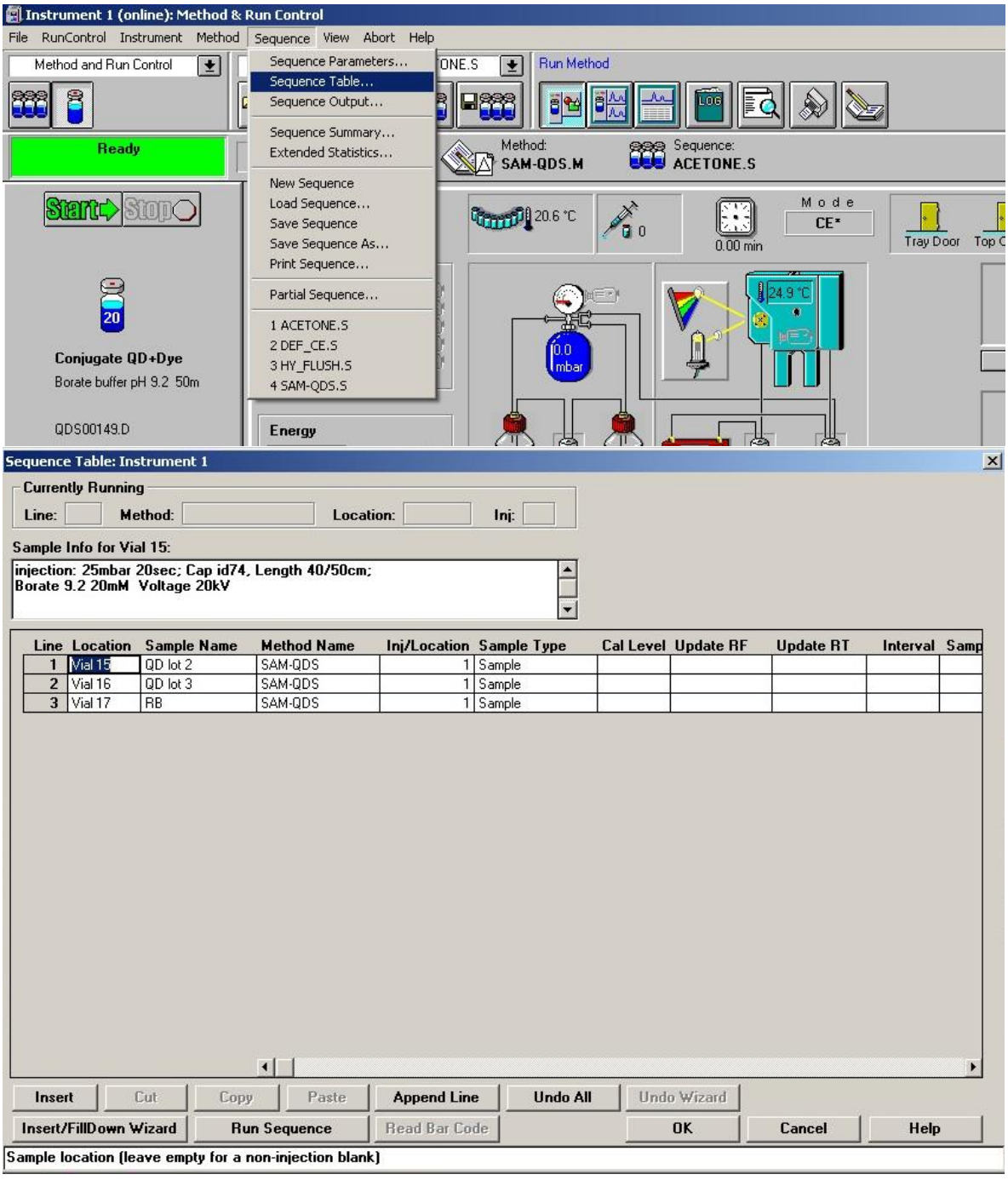


Figure 8 Setting up a sequence

8. Run Control

8.1 Single Run

On the top left corner, choose the “one vial” image for working with single run (Figure 9). Right click the sample tray display to log your sample information, also where you want the data to be stored (Figure 10). Then click Run Method.

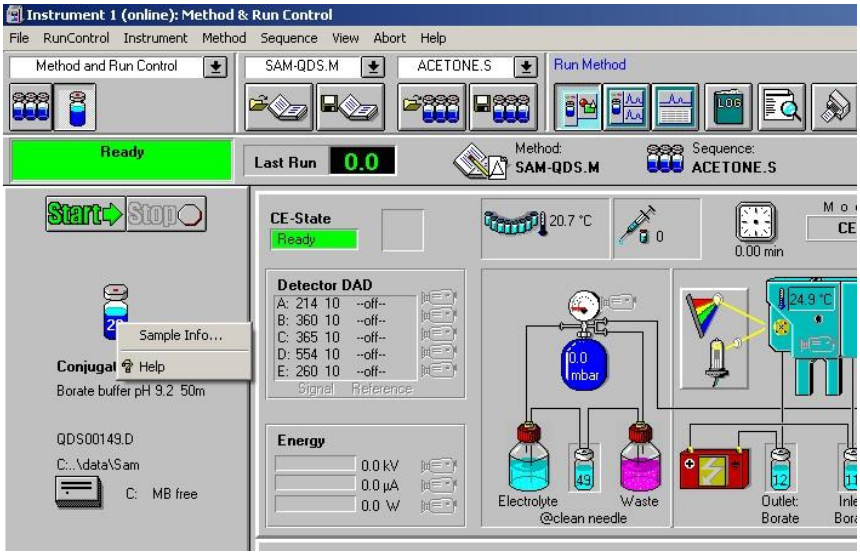


Figure 9. Single Run

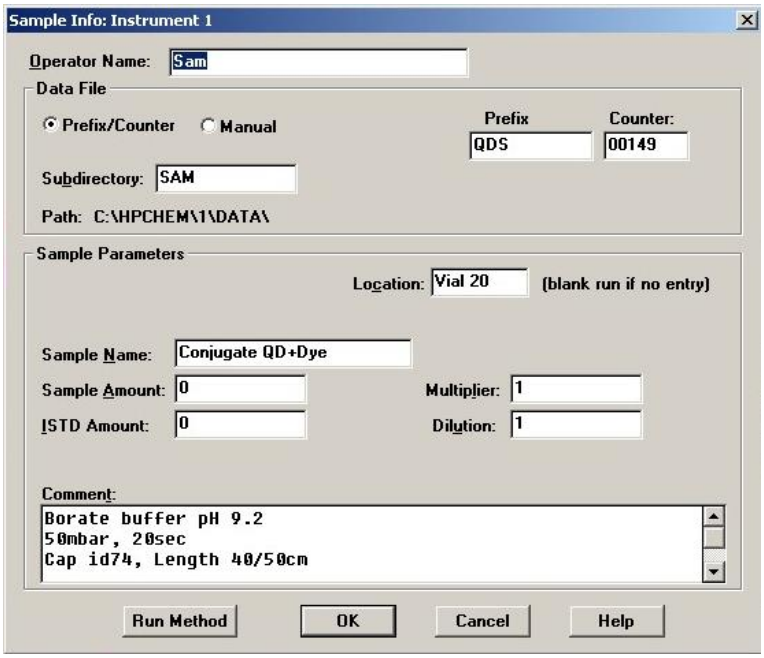


Figure 10. Single Run Sample Info Log in

8.2 Sequence Run

On the top left corner, choose the “several vial” image for working with sequence run (Figure 11). Right click the sample tray display and choose sequence parameters to log your sample information, also where you want the data to be stored (Figure 12). Then click Run Sequence.

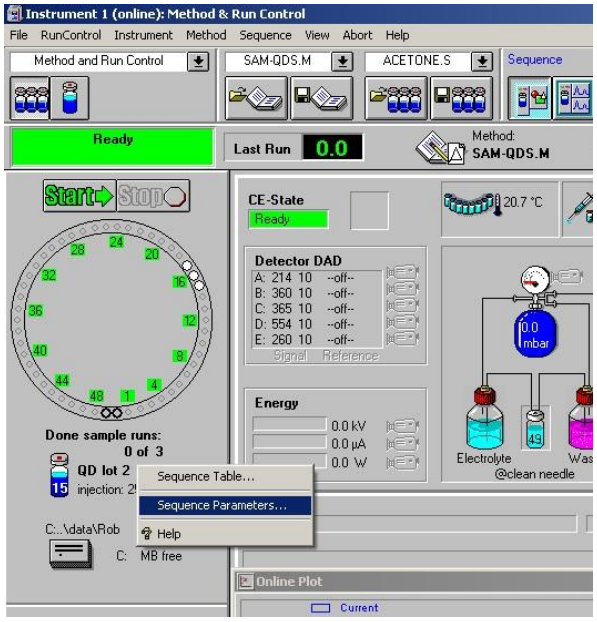


Figure 11. Sequence Run

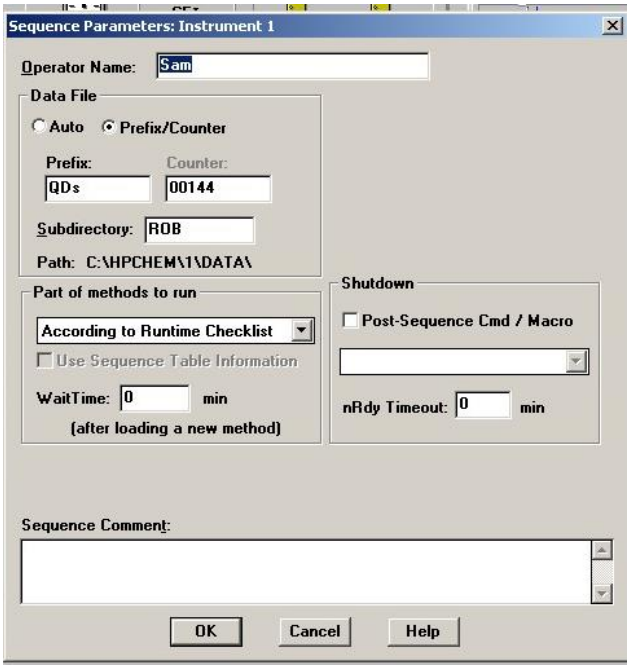


Figure 12. Sequence Run

9. More information on advanced needs

Please refer to the built-in Help system in Chemstation, the hardcopy of manual in the lab or the electronic copy manual online from Agilent for more information or advanced demands.