Capillary Electrophoresis (CE)

November 14, 2017

Instrument instructions can be found at:
http://www.bowdoin.edu/chemistry/instrumentation/instructions/index.shtml

If you have any problems with the instrument or would like to get trained, please contact Celeste Morin
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1) Start-up Procedure

a) Log onto the computer and wait for complete log-on before continuing.
b) Make sure the instrument cover is closed, and push the power button on the CE to turn it on. You will hear the vacuum pump come on. Wait a minute for it to be completely "On" before opening the software.
c) Double-click on the "Instrument 1-Online" icon to open the software program—the orange-yellow LED light will show on the front of the CE initially and then turn green.
d) The software will open into the "Method and Run Control" window. Initialize the instrument by going to – Instrument>System INIT. This calibrates the lamp, sets the cassette temperature and checks tray mobility. The "CE-State" will turn to "Ready" when the initialization is complete.
e) WAIT 20 minutes before the start of a run to let the deuterium lamp stabilize.

2) Tray Set-up—Buffers, Standards, Samples

a) To access the sample tray, left click on the tray icon in the panel and select "Tray Control." (Or go to Instrument>More CE Control>Tray control). This will bring up the direct tray control screen. Click on the "Unload" button, then access the region of the tray that you need to load and unload vials by clicking either "Get Vial" or "Get Segment" to move the tray to where your samples are located. You will need to lower the plastic cover over the tray module to access the tray.

b) As you place your vials into the tray, fill in the table as recommended below. Access this table by clicking on Instrument>System Vialtable. Select the appropriate option in the "Used In" column and type in the "Name" and fill in "Comment" column if necessary. Click "Print" if you'd like a copy of the table for reference, then click "OK."
c) The following locations are recommended:
   i) #1: Buffer-In (home)
   ii) #2: Buffer-Out (home)
   iii) #3: Buffer-Flush (flushing solution)
   iv) # 4: Waste (Make sure the waste vial has 100 uL of water only, let it not be dry or have too much in it.)
   v) #5-8: Standards
   vi) #9: and onward are for samples
   vii) NOTE: #49 can only have DI water, this is for holding the replenish buffer needle (see below-Replenish Buffer Function)

3) Replenish Buffer Function

a) The cabinet below the sample tray has two positions, one for buffer and one for waste. The buffer position has a frit inlet in it. Before each new run, be sure to replenish the two buffer vials (#1 and #2) manually or via the replenish function. You can access the replenish function two ways – but be sure to have enough buffer in the reservoir before you do.
b) Go to Instrument>More CE Vials>Replenish Vial – Enter vial number and Enter height (1.8 cm is the recommended). This function empties out the current contents of the vial and fills with fresh buffer. You will do this for both vials #1 and #2.

OR

c) You can program the replenish function into your method. Replenishing would then be done before the start of the run. (Details in the Method set up).

4) Create/Edit Method

****Note**--All data files, methods, and sequences are stored in the D:\ directory. If creating a new folder for your specific class/research, be sure it is created in D:\DATA, D:\METHODS, or D:\SEQUENCE.**

a) Set up your Method. (Be sure to save often and when you are done with setting up the method). The method set up has several screens. Recommended values are shown below. You set up a method by editing an existing method or creating a new method. Go to Method>New Method or Method>Edit Entire Methods. Going to a New Method will load the default method that you will need to edit by going to Method>Edit Entire Method. For each screen – there is a help button that will define any parameter you are not sure about. After making changes in each window, click on "OK" to move onto the next window. If editing a method, click Method>Save Method to save in current method. If creating a new method, click Method>Save Method As, and type in new name in the correct directory.

b) **1st Screen: Edit Method**: Check Method sections to edit. Check all of them so you can go through all aspects of the method.

c) **2nd: Comment**: Add a comment to describe the method, insert the name of the author and the date created.

d) 3rd: CE Home Values:

i) Offset: 4 mm recommended (this positions the capillary in the buffer)

ii) Cassette: 25C recommended.

iii) Vials

(a) Inlet Home: Select the Buffer vial, usually #1

(b) Outlet Home: Select the other Buffer vial, usually #2

e) **4th: CE Conditioning:**

i) Replenishment and Preconditioning: "Serial" is recommended until you are really familiar with the instrument.

ii) Replenish: Click "None" if you are manually replenishing or "Use Table" if you are programming the replenishing to start at the beginning of the run. If you select "Use Table", then click on edit, and fill in the information for the each of the vials you would like to replenish in a separate line. The information needed is the name of the function "Replenish", Height: 1.8 cm; and Vial Number).

iii) Preconditioning: Often flushing with the buffer for 2-5 minutes is recommended. Some methods will require preconditioning with a different eluent such as NaOH. Click "None" or "Use Table" as above. If using the table, you will use the "Flush" function; enter the number of minutes the flush will take place for, Inlet Vial # (this is the vial that carries the flushing solution) and Outlet Vial # (typically this is the waste vial). Make sure the waste vial has 100 uL of water only. Let it not be dry or have too much in it.

iv) Postconditioning: Again, click "None" or "Use Table". Postconditioning can be used to flush the column after a run with a buffer of your choice. Fill in the table as in the preconditioning above.

f) **5th: CE Injecting:**

i) Select "USE TABLE", and then go to "EDIT".

ii) Injections are based on pressure, and the time for injection is typically the equivalent of the "volume" of injection. Here you specify the details of the injections. The vials to inject are specified in the sequence or set up sample info. (different file). You will need to input function as Pressure: mbar: 50; Secs: 5 (good place to start); Inlet: InjectVial (refers the instrument to the sequence of sample set up info. file); Outlet: OutletHomeVial (refers the instrument to CE Home values defined earlier). In some cases two injections are recommended (so you will input two lines); this post injection plug helps minimize sample loss upon application of a voltage. In this case, you will enter a second line for injection with all parameters the same, except, Secs: for which you will use 2 secs.

g) **6th: CE Electric:**

i) Switch Electric: On

ii) Electric:

(a) Polarity: Positive; Power: Syslimit

(b) Voltage: 30 kV; Current: Syslimit

(c) Lower Alarm Limit: 2uA recommended (drastic decrease in current is a sign of instability). If that takes place, this limit will end your run.

h) 7th: CE Time Table:

i) Store Data: Check "Voltage" and "Current" for sure (and any other parameters important to you).

ii) Time: Stoptime – This is the stop time for the run (how much time you will need for all your compounds to safely elute); Posttime, the time you would like between the current and the next injection. If you do not want any, click "Off".

iii) Time Table: Use in case you are ramping up the voltage, current etc., during the course of the run (avoid doing this until you really understand the CE).

i) **8th: DAD Signals:**
   i) Signals: Specify the wavelengths you would like to monitor; Sample, BW specifies the wavelength range within which the compounds are detected (this will depend on the UV spectrum of your compound); Reference wavelength compensates for baseline fluctuations; use a portion of the UV spectrum of your compound where there is little to no absorption. Reference BW, is the range of wavelengths for the reference.
   ii) Spectrum: Use initially when you are trying to find the spectrum of your compounds of interest. Select "All" to do so and enter the wavelength range. This takes up a lot of disk space so use as necessary.
   iii) Peak Width: This sets the response time for your analysis. Recommended: >0.05 min and Response Time of 1 sec
   iv) Autobalance: Select "Prerun". This zero's the baseline before every run.
   v) Time: input the Stoptime for the run; Posttime: input the posttime for the run
   vi) Timetable: Avoid initially. This is useful if you want to time program your wavelength, etc. during the course of the run.

j) **9th: CE Fraction Collection:** OFF. If you decide to collect fractions, we need to figure out how this works.

k) **10th: Signal Details:** Add the DAD wavelengths that you want monitored during the course of the run. Specify the start and end times.

l) **Edit Integration Events:** We are still figuring out the optimal features. For now let the default numbers be and we can figure out integration in the Data Analysis function, post run.

m) **Peak Top Type:** Unlike LC, CE peaks will be asymmetrical. After a test run, use the help button to figure out the appropriate peak top type for your compounds, and then select the appropriate type.

n) **Specify Report: Instrument 1:** Destination "Screen" will save paper. Style: Select "Short", check "Sample info on each page" and "Add Electropherogram Output", recommended selecting. Other parameters should be left to the default values.

o) **Run Time Checklist: Instrument 1:** Check "Data Acquisition" and "Standard Data Analysis".

5) Create/Edit Sequence

a) A sequence can be created/edited for analyzing many samples. Click on the icon with 3 vials (top left corner of window) to show the carousel on the screen where changes to the sequence can be made. If only one sample will be analyzed, the icon with 1 vial can be selected. Click on the vial picture, the select "Sample Info" to enter the specific sample information and appropriate directory. Click "OK" to save the information.
b) Select Sequence>Load Sequence to load a previously created sequence.
c) Select Sequence>Sequence Table to create/edit the loaded sequence. Make the appropriate additions/changes in the table, and also be sure that the correct method is listed in the table, as the sequence will run the sample based on the method listed in the sequence. When finished creating/editing the sequence, click "OK", then select Sequence>Save Sequence As to save it with a new name, or Sequence>Save Sequence to save it as the same sequence.
d) The sequence table can also be accessed by clicking on the carousel picture and then selecting "Sequence Table". Click "New Sequence" to create a new one. This will automatically delete the current sequence table.
e) **To start the sequence**, click on the "START" icon above the carousel, or, when in the sequence table, you can click "Run Sequence" to start it up.
f) **To start up a single run**, click on the "START" icon above the single vial picture, or when in the "Sample Info" window, click on "Run Method" to save and run the sample.

6) Data Analysis

a) To perform data analysis on completed runs, go to View>Data Analysis, or click on the "Data Analysis" tab in the left column. Load the appropriate data file, and continue with the analysis.

7) Shut-down Procedure

a) Close down the software program.
b) Once the program is completely shut-down, push the power button on the CE to turn it off.
c) Log off the computer.

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8) Other Important Procedures (See Celeste Morin for help)

a) To change buffer in the replenishment system, refer to page 207 of the manual.
b) For long term storage and cleaning of the replenishment system, refer to page 209 of the manual.
c) To install a new capillary in the alignment interface, refer to pages 96-98 of the manual.
d) To install a new capillary into an empty cassette, refer to pages 99-102 of the manual.
e) To remove and insert a cassette into the CE, refer to pages 94-95 and 103-105, respectively, of the manual.

9) Important Notes for Extending Life of the System

a) Capillary ends should be sitting in a buffer when not in use.
b) Make sure there is enough solution in the vial so that the capillary ends are IN the solution so as not to burn the ends of the capillary.
c) Check the ends of the capillary every once in awhile by doing the "Change Cassette" option.
d) Before removing a capillary for storage in a box, it should be flushed with water and then air.
e) EVERY time a new capillary is placed into the CE, flush for 5-10 minutes with 1M NaOH (Agilent suggests at 25C while another paper suggests 60C)
f) Before a series of runs, flush for 2 minutes with 0.1M NaOH followed by 5 minutes of the run buffer.
g) To change the solution in the electrolyte bottle, click on the "Change Bottles" option and then remove the cover. Rinse the bottle out with isopropanol and then place your solution into the bottle.
h) Filter the solution placed into the electrolyte bottle (in plastic case on LEFT) about once a week.
i) EVERY time the solution in the electrolyte bottle is changed, perform the "Clean Tubes" option.
j) If the CE is ever acting abnormally, perform a "System INIT" to reset the system.

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