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 ont o 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
- c) Double- e software program the orange-yellow LED light will show on the front of the CE initially and then turn green.
- d) window. I nitialize the instrument by going to Instrument >System INIT. This calibrates the lamp, sets the cassette temperature and -

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 Or go to Instrument >More CE Control>Tray control). This will bring up the direct tray control screen. C nload button, then access the region of the tray that you need to load and unload vials by

samples are located. You will need to lower the plastic cover over the tho05y9,Nos thea)

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 of ten 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. Afte 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
 - 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) Of f set: 4 mm r ecommended (this positions the capillary in the buf f er)
 - ii) Cassette: 25Crecommended.
 - iii) Vials
 - (a) I nlet Home: Select the Buffer vial, usually #1
 - (b) Outlet Home: Select the other Buffer vial, usually #2
 - e) 4th: CE Conditioning:
 - i) Replenishment a until you are really familiar with the instrument.
 - ii) Replenish: one if you are manually replenishing or able if you are programming the replenishing to start at the beginning of the run. If you sele able 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 eight: 1.8 cm; and Vial Number).

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- 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. Use T using the table, you will use the function; enter the number of minutes the flush will take place for, I nlet 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) Post conditioning: Again, click one or conditioning 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 I njecting:
 - i) Select , and then go EDIT.
 - ii) I nj ections are based on pressure, and the time for injection is typically y the details of the injections. The vials to inject are specified in the sequence or set up sample inf o. (different file). You will need to input function as Pressure: mbar: 50; Secs: 5 (good place to start); I nlet: I nject Vial (refers the instrument to the sequence of sample set up inf o. file); Outlet: Outlet HomeVial (refers the instrument to CE Home values defined earlier). I n 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) Volt age: 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.

- iii) Time Table: Use in case you are ramping up the voltage, current et c., 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.

5) Creat e/ 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

appropriate directory.

he inf or mat ion.

- 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

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

one. This will automatically delete the current sequence table.

- e) To start the sequence
- f) To start up a single run picture, or when in the and run the sample.
- 6) Data Analysis
 - a) To perform data analysis on completed runs, go to View>Data Analysis, or ppropriate data file, and continue with the analysis.
- 7) Shut-down Procedure
 - a) Close down the soft ware program.
 - b) Once the program is completely shut-down, push the power button on the CE to turn it off.
 - c) Log of f t he computer.

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