**Use of the Aviv Model 435 Circular Dichroism Spectrometer**

*Updated by Kevin 3/14/21, CGP 04/02/21*

1. Exposing the instrument to oxygen can **damage the lamp and optics**, which has negative consequences for instrument performance. Always check nitrogen levels before and during experiments.
2. When running experiment, do NOT do anything else with software (f. ex. do not average or look at your data) because the program will likely crash

Starting the instrument

1. Turn on the **nitrogen generator**: follow CAREFULLY instructions posted on the nitrogen generator. The instrument should be purged for at least **30 min.** While the instrument is purging, open the sample compartment, take off the sample holder lid, and take out the sample holder. Place compartment lid back immediately. Check for the spacers in sample holder. They should be pushed all the way in. Use black rectangular prism to push down each sample spot.
2. After 30 min turn on the lamp in the following way:
	1. ALL other electronics should be turned OFF.
	2. Turn on the **XE LAMP PWR** switch.
	3. Wait for the **LAMP READY** light to come on (near ground).
	4. Push the red **IGNITE LAMP** button. Check that DC volts reads ~8. Record hours
3. Let the lamp warm up for at least **15 min** before running experiment. While you waiting set up your experiment:
4. Turn ON the **INST PWR** (Instrument Power Switch) switch.
5. Check water level and start the **Water Bath** (do not adjust anything, just turn ON the switch on the front).
6. Start the computer. Allow the hardware to initialize for at least 120 sec. so that it gets recognized by the computer. After 120 sec start the instrument software.
7. Open the Aviv 435 Icon and wait for the progress bar at the bottom of the program to say “Ready”.
8. Go back to lab and put samples into cuvettes and bring over with cuvette holder. If light sensitive, cover with paper towel.

Set up parameters

1. Go to **control panel**, and select **temperature control**. Set point to 20 degrees C (or desired temperature) and enter. Allow to reach desired temperature.
2. **For wavelength scan**: Use **Configure the Experiment** and under Type select Wavelength scan. Provide Description (basically a note to yourself) and Name (actual filename – include date, sample name, initials). In this window set up bandwidth to 2 nm and temperature to the desired temperature, e.g. 20 C. **Make sure to press enter after setting each number**. Now go to **Experiment Configuration** tab and select **Wavelength Configuration** and set up:
	1. window 330 to 220 nm,
	2. 1 sec avg time,
	3. 1 nm step.
	4. 3 or 5 scans- Use 5 scans for better data, can always stop the experiment after 3 are collected. Use 3 if known good folding
3. **For CD melt:** Use **Configure the Experiment** and under Type select Temperature scan. Provide Description (basically a note to yourself) and Name (this is an actual filename). Now go to **Experiment Configuration** tab and select **Temperature Configuration** and set up the following typical parameters:
	1. Range 4 to 95 °C,
	2. 1 deg step,
	3. 1 deg/min temperature rate,
	4. 15 s avg time,
	5. 0.0833 min (5 sec) equilibration time,
	6. monitoring either 265 nm or 294 nm (but not both).

Important: press Enter after any changes you make.

1. Under **Configure Experiment** - **Save Data options** click on Always Save Experiment to Hard Drive. Click Okay then on **Data Browser** and under **Default Dataset Path** click **Browse** and create the folder that you want to save the data to. Return, return. Followed by **Exit/Save configuration**.
2. At this point, samples should be loaded into the machine. Lift the cover with the black handlebar and remove the black circular top inside as well as the container for the cuvettes. Wipe each cuvette carefully with special non-fiber cloths. Place the cuvettes into the numbered container cells with the cuvette number facing you. Remember which cuvettes were placed in which container cell. Confirm cuvettes are fit in place. The meniscus of each cuvette should be visible from the side of the container. After inserting cuvettes, place the container back into its holder and slowly rotate the container until it clicks in. Place the circular top and cover back on.
3. Using **Control panel** tab, check Rotor Control (make sure that all desired cells are checked); give name for your samples and cuvette number. Then check Temperature Control to make sure it is what you want.
4. Press **Run** experiment.

Running experiments

The following can help you judge how long the experiments will run.

* CD Scans:
	+ One typical CD scan (330-220 nm) usually take ~3.5 min for one sample.
	+ Five scans for five samples should take (5 x 5 x 5 = 125 min, about 2 h).
* CD melt::
	+ CD Melt from 4 to 95 °C and back will take:

|  |  |
| --- | --- |
| **Number of samples** | **Time (hrs)** |
| 1 | 4.5 |
| 2 | 5.5 |
| 3 | 6.75 |
| 4 | 8 |
| 5 | 9  |

Turning off the instrument

1. Save any remaining data
2. Use **File: Terminate CDS Program** to shut down the software.
	1. A last opportunity is given to save any data to the hard drive that may not have already been saved.
	2. The wavelength will move to the home position, causing a delay in the program’s termination
3. Record hours
4. Turn OFF the **XE LAMP PWR** switch
5. Copy or move data off instrument computer.
6. Shut down the **Windows** software. (Wait until shut-down is complete).
7. Turn OFF the **Water bath.**
8. When shut down is complete, turn OFF the **INST PWR** switch.
9. Wait 10 minutes after shutting off the lamp before turning off the nitrogen flow. Turn off the generator (see instructions on the generator for turning it off).

Normally we average data using Origin, but you can also average using CD instrument software in the following way:

**Averaging of the data for wavelength scans (right after an experiment)**. Once experiment is completed you can perform averaging in the following way. Axis definition→Data collection av (check traces that apply)→Av. selected traces→ok. Now change file name (for example, add “\_av” to the existing name)→save av. trace→yes→ok→return. At this point you should see a white line; that is your av. trace. Make sure it looks fine to you. Go to Display→data. Save data via Data Browser

**Averaging of the data for wavelength scans**. Open up view-only mode of software

* 1. File→load data set→read data set **from** disc (this loads data from disc to the program). Locate your dataset and then load it.
	2. Displays→data review→wavelength (this sets the view window to the appropriate mode)
	3. Axis definitions→left multi data set→then select your datasets from the list
	4. Axis definitions→data review average→select all traces
	5. Rename experiment (by adding av), then save average trace (click yes when it asks you to create a new experiment). Return
	6. Perform this for each experiment of interest. To clear graphs off the screen, select axis definitions→clear left axis