Circular Dichroism SOP

By Melissa Sprachman

I. Startup

1. Sign the logbook.

2. Open the main valve of the nitrogen cylinder and then open the needle valve. Check the nitrogen gauge (left side of the instrument). The reading should be between 20 and 30. You may need to adjust the needle valve to attain this reading. **Do not change settings on the regulator or the gauge itself.** Alert Melissa if there are problems.

Note: Check the tank pressure before proceeding. If the pressure is less than 500 psi, the tank will need to be changed prior to running experiments. It is critical that you pay attention to the nitrogen reading throughout the course of your experiment!!).

3. Power on the instrument (main green power switch at the lower left of the instrument).
4. Ensure that the silver selection knob at the right of the instrument is set to "CD" or "ORD" according to which experiment you will be running.

5. Click icon for "CD User" and enter the username and password.

6. Start the **Spectra Manager Program** and select an experiment (typically "Spectrum Measurement" for acquiring CD spectra).

7. A warning regarding the nitrogen purge will appear. Allow the timer to count-down the 5 minute purge time. The lamps ignites at the end of the countdown. **Note:** A series of self-diagnostic tests are performed. Wait until the diagnostics are completed. If an error message appears, select "Try again." If the second ignition attempt fails, contact Melissa.

8. Allow **30 minutes** for the lamp to warm-up prior to beginning experiments.

9. If the peltier temperature controller will be used, turn on the water bath, which is set at 20 ºC. (Note: There are two power switches on the bath—one on the bath portion, one on the control box.)
   NOTE: Do not allow water to circulate when the lamp is not lit!

II. Data Acquisition

1. On the drop-down menu bar, select Measurement | Parameters to set the parameters for your experiment.

a) Select the "Parameters" tab. The following are only a guideline for peptide/protein solutions. Guidelines for small molecules are in parentheses if they are different than protein guidelines.

(1) Bandwidth = 1 nm.
(2) Response = 4 sec (1 sec)
(3) Sensitivity = Standard.
(4) Measurement Range = 255 – 190 nm.
(5) Data Pitch = 0.1 nm.
(6) Scanning Mode = Continuous.
(7) Scanning Speed = 100 nm/min.
(8) Accumulation = 5.(1)

2. At the "Data Mode" tab, ensure the settings are appropriate for your experiment:

For CD experiments: Channel #1 = CD
Channel #1 = HT

For ORD experiments: Channel #1 = ORD
Channel #2 = HT

3. Select the "Data File" tab to increment and autosave files as they are acquired. If the boxes are left blank, make sure to save each file as it is completed.

4. Select the "Option" tab to enter sample-specific data. This information will be printed on your results.

5. Click [OK] to apply the parameters and close the dialog box.

2. If the Peltier device is in use, set the temperature under Select Measurement | Accessory then close the box.. Press "START" on the Peltier control panel to begin bringing the device to the appropriate temperature.

3. Click [Start] to begin data acquisition.

III. Data Processing—these tasks can be performed in the "Spectral Analysis" program

1. If you selected "auto save," a window containing data for your run should automatically appear.

If autosave was not enabled, then save the data under File |Save. This data file will contain mdeg and HT.

A. Blank subtraction:

1. Correct the analyte data by subtracting the blank. **The blank must be collected using the same parameters as the analyte spectrum**

2. With your analyte spectrum ope, select Processing|Subtraction. In the window that appears, there is an option to open another data file. Open the file containing the blank. The top line in the window shows the order of subtraction. It should indicate that the blank is being subtracted from the analyte file. If the two are reversed, press [Exchange]. Press [Apply]. Save the results under File|Save.

B. Converting HT data to OD data:

Select Processing -> CD Options -> HT to OD. Use this window/file for all remaining processing steps.

C. Convert from CD mdeg to mean residue ellipticity:

Select Processing - > CD options -> Optical constant

Selection [Molecular]. The "Specified Channel" should be "Ch. 1 CD(mdeg). Leave Channel 2 blank.

Enter the cell length and concentrations. You do not need to fill the "molecular weight" or "magnetic field" boxes.

Press "OK" and save the resulting spectra as a new file.

Note: The files are saved as .jws files, which are only readable using the Jasco software. It may be more useful to export your files as ASCII text files (you can do so in the file/save menu).

IV. Shutdown.

1. Make sure all data (and corrected data files) are saved.

2. Shut off the water bath (Peltier unit) if it was in use.

3. Go to Control -> Light Source and uncheck the box next to "Lamp" to turn off the lamp. A box will appear telling you to stop the nitrogen purge. **Purge the instrument with nitrogen for 5 minutes after shutting off the lamps and then turn off the nitrogen.**

4. Close the Spectra Manager program, turn off the main green power switch, and turn off the temperature bath (if in use). You should perform these operations while the instrument is being purged.

5. Remember to sign-out of the logbook.