

Basic Operation of Jasco J-810 Spectropolarimeter

I. BEFORE YOU START

OPERATION OF THE CD REQUIRES A CONTINUOUS FLOW OF NITROGEN.

The Xe lamp converts air to ozone. Ozone will damage the mirrors forcing costly repairs. ☹

Without nitrogen flowing, you can completely fry the optics in less than an hour!

It is your responsibility to make sure you have enough N₂ for your experiments. If the tank is less than 1/3 full, please inform Laura or Heather immediately so that we can order a new one.

You must increase the N₂ flow rate if you are measuring at shorter wavelengths:

200-185 nm: 20-30 ft³/hr

185-180 nm: 30-45 ft³/hr

Below 180nm: ~100 ft³/hr (maximum)

NOTE: Molecular oxygen absorbs in the far UV and decreases the amount of light that gets to your sample, so having a higher N₂ flow rate can help with sensitivity as you scan to shorter wavelengths. The practical limit of our instrument is 185nm for biological samples in aqueous buffers.

II. START UP

1. Open valve on N₂ tank. The flow meter attached to the instrument should read ≥ 20 ft³/hr, higher if you are scanning below 185nm (see requirements above). The level is read at top of silver indicator bead.
2. Purge the optical system. It takes ~5 min to displace all the air if N₂ flow = 20 ft³/hr. You MUST do this before lighting the lamp!
3. Sign the logbook.
4. Log onto computer with your VUNet ID and password.
5. Turn on CD: ON/OFF toggle switch is on front of instrument.
6. If applicable, turn on Peltier temperature controller. ON/OFF switch is on front of controller. Usable range: -10 to 110°C. You MUST turn on a flow of water. This is required as a heat sink for Peltier cooling. The flow should be a steady trickle. *Do not open the valve all the way as the water pressure could burst the tubing and cause a flood.*
7. Launch the 'Spectra Manager' program from desktop icon.
8. Starting a measurement program will automatically light the lamp (green indicator on front of CD will light). NOTE: Turning the lamp ON/OFF frequently will shorten its life, so it's better to leave it on until you are done for the day.
9. Check the # of lamp hours: [Control] ⇒ [light source]. Record number in logbook.
10. Check that the high tension voltage HT[V] ~250. If it's near zero, it means that light is not hitting the detector. Remove the black sample spacer (if it's in the sample block) and make sure that the lid to the sample compartment is fully closed. NOTE: If you see HT[V] steadily increasing with no sample in the instrument, there's a problem with the mirrors or the lamp—please inform Laura immediately.
11. You can begin taking data right away, but for important measurements, it's best to wait until the instrument warms up (takes ~30 min.).

NOTE: Spectra Manager can only run one measurement program at a time.

III. COLLECTING DATA

I've collated some information on recommended buffers, concentrations, cell pathlengths, etc. on a separate sheet entitled *Notes on CD sample preparation*. You can download this from the CSB website: <http://structbio.vanderbilt.edu/wetlab/cd.sample.prep.php>

The CSB's cuvettes are used by lots of people, so it is a good idea to check that they are clean prior to use. Squirt some water into a DRY cuvette. It should flow easily down the sides. If it sticks or forms air pockets, it means that the cuvette is dirty. Follow the posted cleaning instructions. If the cuvette is still dirty, then see Laura or Heather.

1. Place sample cuvette in chamber.
 - When switching samples, minimize the time the sample compartment is open.
 - Be consistent with cell orientation.
 - Use the black metal spacer with 1mm cell. NOTE: You can use the hole in the spacer as a gauge for how much sample you need: solution in cuvette must cover this hole completely.
 - If using temperature controller with 1mm cell, place cuvette to the left of spacer on the side closest to the external temperature probe.
2. Double click on a desired program from 'Measurement' menu.
3. If you are using Peltier temperature controller, you must select it as an accessory: [Measurement] ⇒ [Accessory], otherwise the computer can't communicate with it. You can change the setpoint temperature from the computer [Control] ⇒ [Accessory], or using keys on the controller. The [START] button on the controller must be lit for the temperature controller to work.
4. Run a quick survey scan to make sure your sample concentration is in the right range. See NOTE below and section IV.
5. Enter desired parameters: [Measurement] ⇒ [Parameter]. See Section IV for more on parameter selection.
6. Go to the Options tab to enter sample conditions, operator name, etc.
7. Go to Data file tab. Checking the 'Autosave' and 'File Serial #' boxes will save a series of files under the file name you enter. Create your own personal folder on the network J: drive for your files. Data saved to the J: drive are backed up on a nightly basis.
8. If using the Peltier accessory, wait until temperature is equilibrated before starting measurement. You cannot alter anything once acquisition has begun.
9. On completion, the data automatically transfer to the analysis program. If you want to export the data to another program (i.e., secondary structure deconvolution programs), use 'Save As' to save a copy of the data in *ascii* format. If you save your data on the J: network drive, it can be accessed remotely. Follow the instructions given at:
http://structbio.vanderbilt.edu/wetlab/private/shared_drive_access.php
10. Clean the cuvette and run your sample buffer under the same conditions you used for your sample.

NOTE: The instrument adjusts the high tension voltage in order to maintain a constant DC component of the photomultiplier tube. The data are unreliable when HT[V] exceeds ~650-700. Usually, when the HT[V] goes out of range, you'll see a corresponding discontinuity in your CD data.

IV. PARAMETERS

For more details, consult the appendix of the *Model J-810 Spectropolarimeter Operation Manual*, p. 61 and the *CD-ORD Measurement Manual* p. 14.

Checking Protein Concentration: It's a good idea to do a fast spectral measurement first to check sample concentration. Here are some suggested parameters for a quick survey scan:

Sensitivity: Standard (100mdeg).

Wavelength range: 260-190 nm for far-UV scan; 350-250nm for near-UV scan

Far-UV measurements are limited by buffer and sample absorption. A smaller pathlength cell can help extend the lower limit of your spectral range.

Data Pitch: 1nm

Scanning Mode: Continuous

Scan speed: 100 nm/min

Response: 1 sec

Bandwidth: 1nm

Accumulation: 1

You should have a spectrum with a maximum in the range of 20-50 mdeg (absolute values), and a HT[V] < 650-700. If the signal is too intense or too low, adjust the protein concentration accordingly and repeat the acquisition. Diluting the sample will often improve your ability to scan at shorter wavelengths.

Optimizing Parameters: Here are a few things to keep in mind.

Sensitivity: Set this so that your data stay on scale. The Standard setting sets the range to $\pm 200\text{mdeg}$. For near-UV spectra where the signal is typically weak, you may need to use the High setting.

Spectral Range: CD always starts at longer wavelength and ends at shorter. You'll get an error message if you reverse the order of the endpoints. Set your end wavelength at a point where $\text{HT}[V] < 650-700$. (See *CD-ORD Measurement Manual* p. 13, bottom.)

Data Pitch: Increments at which you take data points. Make resolution coarse if peaks are broad, fine if peaks are sharp.

Scanning Mode: Jasco rep said to leave on 'Continuous' unless you really know what you are doing.

Scanning Speed: This parameter is coupled to Response (see below).

Response: Data at a given point are the average value of sampling before and after that time ($\text{Response}/2$). The longer the Response, the greater the effect of previous data on the current data. You do not want to increase Response when Scanning Speed is high or when Data Pitch is low. The Jasco rep. said that as a general rule:

$$\text{Scanning speed} \times \text{Response} \leq 200$$

Bandwidth: A measure of the precision with which a monochromator selects light of a chosen wavelength. Increasing the bandwidth allows more light to hit the sample but decreases the ability to resolve fine structure.

Accumulation: Number of measurements that are averaged. Increasing this improves the S/N but also prolongs measurement time. Baseline drift is a factor with longer experiments.

IV. PARAMETERS (cont.)

Good Spectral Scan: Here are some example parameters I culled from various web sites.
For more information see Jasco Operation Manual Appendix 9.1 'How to Select Parameters'

	Far-UV	Near-UV
Wavelength range:	260-190nm	330-250nm
Data Pitch:	0.5-1nm	0.2-0.5nm
Scanning Mode:	Continuous	Continuous
Scan speed:	20, 50 nm/min	10, 20 nm/min
Response:	2-4 sec	4 sec
Bandwidth:	1-2nm	0.5-1nm
Accumulation:	1-2	3-5

Temperature Melt: Here are some example parameters for thermal denaturation with a 1mm cell.
Use the Teflon stopper for the cuvette to avoid solvent evaporation.

Wavelength: Pick a wavelength where folded/unfolded spectra differ significantly. For alpha helical proteins this is usually 222nm.

Start: 2-5°C

Stop: 95°C

Data Pitch: 1-2°C

Delay Time: 1.5-3 min. This is the initial delay after the set temperature is reached that allows the sample to equilibrate before the melt starts.

Temperature Slope: 40-60°C/hr. This is how fast the temperature is raised during the melt.

Response: 4-8 sec

Bandwidth: 1.0nm

V. SHUT DOWN:

1. Make sure all your files are saved in a personal folder on J: network drive.
2. Check the # of lamp hours: [Control] ⇒ [light source]. Record number in logbook.
3. Uncheck the LAMP ON box. Click OK. This will turn off the lamp. A splash screen should appear which says to turn off the nitrogen. Close the main valve on the nitrogen tank.
4. Exit the software. Do this **before** turning off the CD otherwise the software will freeze. If it freezes, hit <Control> <Alt> <Delete>. Under the 'Task Manager' tab, you can force quit Spectra Manager.
5. Turn off CD. If applicable, turn off temperature controller and water flow.
6. Remove your sample and clean the cuvette.
7. Log off computer and turn off monitor.