

Jasco J-810
Spectropolarimeter

Basic Operating Manual

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This manual serves as a short-course guide to the basic operating procedures to measure CD spectra, the most common maintenance, and the most frequent troubleshooting issues for the Jasco J-810 circular dichroism spectropolarimeter.

A very important note to consider when comparing multiple spectra is concentration. It is absolutely critical that concentrations are accurately determined. Otherwise, comparison between samples is futile.

For proteins, it is recommended that the ellipticity signal is converted to mean residue ellipticity. This corrects for the number of peptide bonds and concentration as a pseudo normalization process for comparison between mutants with their wild type predecessors as well as comparison between different proteins. Mean residue ellipticity can be calculated as follows where θ_{obs} is the observed ellipticity signal, n_{pb} is the number of peptide bonds (# amino acids – 1), l is pathlength and c is concentration.

$$[\theta] = \frac{\theta_{obs}(\lambda)}{n_{pb} \cdot 10 \cdot l \cdot c}$$

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I. Basic Instrument Initialization:

a. Purging with N₂ Gas

You should find the instrument located in the Biochemistry building in room 3517. The instrument consists of the main spectropolarimeter unit with the monochromator on the right side of the sample chamber, a Peltier temperature controller (Model JWJTC-484), a water bath, and Dell computer. Also integral to the instrument is a nitrogen gas line connected to N₂ cylinders at the end of the bench.

To use the instrument, use the log book to reserve the instrument, track user history, and monitor lamp life.

First, before turning on the instrument, be sure to purge the instrument with nitrogen gas for 10 minutes. This is accomplished by turning the tank regulator anti-clockwise. There are four ways to regulate the pressure of the gas. The first is the tank regulator which is part of the gas tank itself. Second is the large black knob facing out on the Fisher Scientific gas regulator. The third is the small black knob facing down on the Fisher regulator. The final way is the gas flow regulator directly attached to the front of the sample chamber. If nobody changes the settings on the other knobs, the only thing the user will need to do is open the flow of the gas directly from the tank. Here, a full tank should have pressure between 2000 and 2700 psi. Tank pressure can be monitored by the pressure gauge on the right. The large black knob controls the flow rate through the Fisher regulator and should read between 8 and 10 psi, as viewed on the left gauge on the Fisher regulator. The sample chamber regulator should have the top edge of the floater at 40 psi. The approximate flow rate is 450 liters per hour. Thus, a full tank should be

able to last for 4.5 – 6 hours. It is critical that the instrument never be run without nitrogen gas. The optics can be ruined from the buildup of ozone on the optical mirrors if run without nitrogen. **NEVER OPERATE THE INSTRUMENT WITHOUT NITROGEN GAS. The user should replace cylinders no later than when the pressure on the Fisher regulator reads 200 psi.**

It is important for the user to replace nitrogen cylinders as they use them to ensure that enough gas is available for future users. Room 3517 has the capacity to store 4 cylinders at a given time. One should always be securely connected to the instrument via the bench-top cradle, while three can be securely stored with a chain along the wall. It may be necessary to use Teflon tape to prevent gas leaking between the tank and regulator.

Typically, empty cylinders are securely stored w/ lids off to indicate that they are empty. However, empty cylinders should be replaced as soon as possible and not left for the next user to deal with. Replacement cylinders can be exchanged in the chemistry receiving department. Users should use ‘general use’ as the user name and account number 111640GE when checking out cylinders. Be sure to sign and date the card when checking out and returning cylinders.

b. Turning Jasco J-810 Instrument On/Off

To turn on the CD spectropolarimeter after purging with nitrogen gas, make sure the computer and monitor is turned on. The default username is ‘Administrator’ while the password is left blank. Just click ‘OK’ to proceed and log in (Note: It is not necessary to turn off the computer between usages. Just log out and power off the monitor). Next, turn on the instrument by flipping the large green switch on the front of the spectropolarimeter to the ‘On’ position (You will hear an audible beep). Next, turn on the Peltier temperature controller and the water

bath. If the user will be measuring spectra at very low temperatures, change the water bath temperature to 14 degrees C while measuring at these low temperatures. The normal temperature setting for the water bath should be 18 degrees C. The instrument is now on and ready to be initialized.

c. Initializing the Instrument

Double click on the 'Spectra Manager' icon on the computer's desktop. A menu of software program macros will appear. For a simple far-UV or near-UV measurement over a given wavelength range, double click on 'Spectrum Measurement.' The instrument will now undergo its initialization process by checking the internal diagnostics (See troubleshooting guide if errors occur). Once the diagnostics are completed, the shutter will open and the lamp will be turned on. Confirmations of these processes are both audible by single clicking noises and visual by the lumination of two green lights on the front panel of the instrument. The lamp should be allowed to warm up for ten minutes. When the lamp has reached 400+ hours, allow the lamp 15 – 20 minutes of warm up time to ensure reproducibility. **The lifetime of the lamp is around 500 hours. It is imperative that the user record the lamp life before and after their experiment.** To accomplish this, select 'Light Source' from the 'Control' menu. In the log book, record the lamp's hours both before and after the experiment.

d. Using Peltier Temperature Controller

If the user wishes regulate the temperature of the sample for reproducibility and/or to take measurements at various temperatures, the Peltier temperature controller must be activated. To do this, in the Spectrum Measurement program, click on the 'Measurement' menu, then 'Accessory.' Select 'JASCO Peltier Type (Single, RTE On)' in the 'Temperature' drop down

menu. Then, click ‘OK.’ The temperature controller will now be initialized. **To activate the temperature controller, the user must press the ‘Start’ button on the front of the temperature controller.** If the user does not press ‘Start,’ the Peltier unit and the software will not synchronize. To change the temperature, select the ‘Control’ menu and then click ‘Accessory’ and the accessory control panel will appear. Key in the desired temperature and click ‘Apply,’ then ‘Close.’ The temperature controller will now change to the desired temperature.

e. Summary of Initialization

1. Reserve the CD instrument ahead of time using the logbook
2. Purge CD with nitrogen gas for 10 minutes
3. Ensure computer is turned on, and then turn on instrument, Peltier, and water bath.
4. Initialize the instrument and record the lamp lifetime in the log book
5. Initialize the temperature controller using the computer software and physically press ‘Start’ on the temperature controller.

II. Spectrum Measurement

a. Parameters

To collect CD spectra, click on the ‘Measurement’ menu and select ‘Parameter.’ Here is where the user can change the parameters to best suit their needs depending on the sample to be measured. **It is recommended that each user consult the literature for appropriate**

parameters and to have a good idea about what to expect from the signal output. Under no circumstances should the instrument be used without a good frame of reference.

Each sample is different. The utmost consideration should be given to sample composition. It is best to keep the sample composition as simple as possible. Many buffers absorb (any chiral molecule can have CD) and protein or DNA concentration greatly affects the signal to noise ratio. These are perhaps the most important things to consider when preparing samples. Again, consult the literature before using the instrument.

The basic parameters generally used are as follows:

Sensitivity: Standard (100 mdeg)

Start Wavelength: 250 (far-UV CD), 320 (near-UV CD)

End Wavelength: 190 (far-UV CD), 250 (near-UV CD)

Data Pitch: 1 nm

Scanning Mode: Continuous

Scanning Speed: between 10 – 100 nm/min → slower scanning rate generally gives better data quality

Response: this parameter depends on the scanning speed. These two parameters are inversely proportional. The response time is a pseudo averaging time which allows the data to stabilize.

Bandwidth: 2 nm

Accumulation: 1 → Accumulation determines the number of scans. Typically, multiple scans are taken at higher scanning rates and these are manually averaged. Using a slower scanning rate can alleviate this.

Under the ‘Data Mode’ menu, the user can select up to 4 “channels.” Typically, only two are necessary. Channels 1 and 2 should always monitor CD signal and HT (Voltage) respectively. It is imperative that the user monitor the HT signal as this gives a direct measure of the amount of light being produced and passed through the sample. If the voltage increases above 700, the signal will start to become noisy and the CD output will become unreliable. If the voltage increases beyond 800, the noise will become disproportionate to the sample signal and the output will be completely unreliable.

The voltage increases proportional to the absorption of the sample. If a buffer component is highly absorbent of circularly polarized light, the light source will increase the amount of light being generated to achieve the same level of light passing through the sample (not absorbed). If the voltage raises beyond 700, the user should dilute the buffer concentrations accordingly. **It is a good idea if the user is unsure of the absorptive properties of their buffer to run a buffer blank before preparing samples to assess the extent the buffer contributes to the CD spectrum. Be sure to check absorbance over the range of wavelength you intend on using.**

Voltage, thus absorbance, is wavelength dependent. To ensure that the sample does not max out the limitations of the detector, insert your sample in the sample chamber and close the lid. Then, select the 'Control' menu and select 'Move Wavelength.' Change the wavelength to the ends of the wavelength range you wish to monitor each time allowing the data to stabilize and monitor the voltage. This can be viewed from the spectrum measurement window viewing the digital box on the second row down, third from the left. Repeat this with a few wavelengths in the middle of the range. If the voltage is below the noise threshold, continue with operation (Note, voltage increases with increasing temperature. If you wish to do a temperature/wavelength scan, be sure the voltage never exceeds 500 volts at the starting temperature).

Once again, referencing the literature is the best way to determine a starting point.

Once the parameters are input and the voltage checked over the wavelength range, select the 'Data File' menu from the 'Parameter' menu. Give your spectrum an appropriate name and save it accordingly in a folder under your name.

The 'Option' menu allows the user to record important information regarding their sample. This is an optional feature.

b. Measuring and Saving CD Spectra

You may now push 'Start' on the spectrum measurement window. The spectrum will be obtained and a new 'Spectra Analysis' window will appear showing your spectrum. To save this file as a text document for further processing using signal processing or spreadsheet software, select 'File' from the menu, and then 'Save As.' Select the appropriate folder and make sure the file extension .txt is used. This file can now be transferred to your computer and processed further.

c. Summary of Spectrum Measurement

1. Input appropriate parameters
2. Check wavelength dependent voltage of buffer and sample (not to exceed 700 volts)
3. Give spectra name and direct it to save in appropriate folder
4. Take measurement by clicking 'Start'
5. Save file as text document for further processing

III. Other Programs

a. Time Course Management

This program monitors a single wavelength over a period of time. The time course program is a way to measure kinetic processes on the timescale of seconds to minutes. As it only monitors at one wavelength, it is important that the sample measured absorbs strongly and consistently at a characteristic wavelength.

b. Interval Scan Measurement

c. Data Monitor

The data monitor program is not generally used for spectral measurements. This program is used during calibration and to monitor the signal when performing the CD scale check after replacing a lamp.

d. Environment

This program is only used when performing calibrations during preventative maintenance by Jasco technicians. General users do not need to use this application.

e. Variable Temperature

The variable temperature application allows the user to monitor a single wavelength as a function of temperature. If a single characteristic absorbance is observed in a sample, this program will allow the user to perform thermal denaturation experiments monitoring at that single wavelength.

f. Temperature / Wavelength Scan

The temperature/wavelength scan is an extension of the variable temperature program. This application features the ability to monitor a range of wavelengths (ie far-UV, near-UV -- like the spectrum measurement program) and a single wavelength as a function of temperature. This program allows the user to monitor the entire spectrum of interest as a function of temperature which is especially useful when measuring samples which have multiple wavelengths where the sample absorbs circularly polarized light. This program allows the ability to monitor complex relationships and coupling between various wavelengths for more thorough analysis compared to following a single wavelength. Use of this program is nearly identical to the 'Spectrum Measurement' application. The only difference is that there are two variable parameter menus to navigate (temperature & wavelength). Note: to change the range of

temperatures, enter the desired starting and final temperatures in the ‘Temperature’ menu, then click the ‘Reset’ button followed by the ‘Append’ button.

g. Titration Spectrum Measurement

Refer to the ‘Automatic Titration System Instruction Manual’ for more detailed instructions and schematics of the titrator module. The model number of the module is ATS-429S/429L.

The titration programs utilize the optional titration module and are a simple way to perform chemical titrations. A 1 cm cuvette must be used with the optional cuvette cover and appropriate attachments. Assembly of the cuvette is self explanatory, and the tubing delivering the solute injections should be routed through the black circular protrusion at the top of the sample chamber lid. There are two holes drilled through to the sample chamber for the inlet and outlet flow of solution. **Extreme caution should be considered when using salts which will crystallize and cloud the optics if the sample volume exceeds the capacity of the cuvette at any time.**

It should be noted that in the experience of this user, the internal calculations performed using the software are incorrect. It is prudent to perform dilution and concentration calculations manually instead of relying on the software calculations. It is also necessary to check the volume of the injections. With CD, concentration is a critical variable that propagates large errors if not checked.

The titration spectrum measurement program monitors a range of wavelengths with each injection of solute. Chemical denaturation or ligand binding are common measurements monitored with this application.

h. Titration Measurement

Titration measurement simply monitors a single characteristic wavelength with each injection of solute. See notes above for precautions.

IV. Maintenance

a. Changing Lamp

The Jasco J-810 uses an Osram 150 watt Xenon lamp (**Jasco Part Number 3027**) which has a typical lifetime of 500 hours, but the lifetime can vary between new light sources. It is recommended to keep a spare lamp on hand if the user notices much more noise than usual, having to wait for longer than 20 minutes for the signal to become steady, or if the initialization diagnostics upon instrument startup indicated that the voltage (HT) is “Too High.” It is a good idea to regularly monitor the voltage with no sample in the sample chamber at 250 nm and to record the voltage at this wavelength in the logbook upon replacing the lamp. An indication of the lamp being replaced should also be noted in the logbook. If the voltage increases more than ~ 50 volts, try replacing the lamp.

It is critical for safety that the instrument is turned off before replacing the lamp. It is also HIGHLY recommended that if the instrument has been on before lamp replacement, the light source should be thoroughly cooled before handling. The lamp anode and cathode holders should be cool to the touch. **Never handle the lamp by touching the glass.**

The orientation of the lamp is critical. **The polarity of the Xe lamp should not be confused. Be sure the anode end is on top and the cathode on bottom.** A “knob” is apparent in the middle of the glass “bulge.” **This should never face either of the mirrors (M_0 & M_1).** If the voltage is “Too Low” when initializing the instrument for the first time after lamp replacement, check the orientation of the lamp. Being misaligned by a millimeter can have a huge affect on the voltage. (See pages 23-25 in the ‘Hardware/Function Manual’)

Follow the steps below to replace the lamp:

1. Ensure the instrument is OFF
2. Remove the light source cover on the back left corner of the top of the instrument.

This is a “mesh” covered enclosure fastened with three fixing screws.

3. Loosen the two screws which hold the light source in place. These two screws are tightened against the anode holder.

4. Slowly remove the light source by holding the anode holder and unscrewing it from the light source. **The light source is spring loaded so use extreme caution and have a steady grip at all times. Also make sure to note the orientation of the protrusion on the bulge of the light source so you know which way to reinstall the new light source.** The anode holder is the metal end which is used to pull the light source from the chamber.

5. Remove the cathode adaptor from the old lamp. The cathode adaptor is the slightly pointed metal end at the bottom of the light source.

6. Attach the anode holder and cathode adaptor to the new light source. **Do not touch the glass of the light source.**

7. Secure the light source via the anode holder with the two fixing screws making sure the protrusion on the glass bulge of the light source does not point towards the mirrors. It is best to replace the light source with the protrusion in the same orientation as the old lamp.

8. Secure the light source cover and with the three fixing screws.

9. Start up the instrument

10. Initialize the instrument by opening the data monitor program

11. Select the ‘Parameter’ command from the ‘Setting’ menu and set the following:

Sensitivity: Standard (100 mdeg), Response: 1 sec, Band Width: 1 nm.

12. Select the 'Data Mode' command from the 'Setting' menu and input the following:
Num. of Channel: 2, Channel #1: CD, Channel #2: HT.

13. Select the 'Setting' menu and 'Move Wavelength' to 546.1 nm.

14. Remove the cover of the M_0 and M_1 mirrors (see page 25 in 'Hardware/Function Manual for illustration) and see 3 adjustment screws for each mirror.

15. Adjust the screws for the M_1 mirror to minimize the voltage with the sample chamber empty. This takes patience and very fine adjustments. **Typically a HT value to try and achieve is 310 Volts at 546.1 nm through adjustment of the M_1 mirror.** The manufacturer suggests a value between 200 – 300 volts, but this user has never achieved anything below 300 (the Jasco technician could not either).

16. Adjust the screws for the M_0 mirror to minimize the voltage with the sample chamber empty. **It is the experience of this user that the M_0 mirror needs minimal if any adjustment. Focus on the M_1 mirror.**

17. Reinstall the mirror covers

b. CD Scale Check

Once the lamp has been replaced, it is necessary to adjust the CD scale to ensure proper amplitude of the CD signal. Follow the procedures below:

1. Start up the instrument and initialize the 'Spectrum Measurement' program
2. Allow the lamp to warm up for 1 hour prior to adjusting the CD scale
3. Prepare a 0.06% (w/v) aqueous solution of ammonium d-10-camphor sulfonate. This is achieved by weighing out 0.03 g (30 mg) and dissolving in 50 mL. **It is very important to make an accurate sample as reproducibility will be influenced between lamp changes otherwise.**

4. Select 'Parameter' from the 'Measurement' menu and set parameters to the following: Sensitivity: Low(1000mdeg), Start: 350 nm, End: 250 nm, Data Pitch: 0.1 nm, Scanning Mode: Continuous, Scanning Speed: 50 nm/min, Response: 1 sec, Band Width: 1 nm, Accumulation: 1. Under the 'Data Mode' menu, select 2 channels, CD and HT respectively.

5. Fill a 1 cm pathlength cuvette with the 0.06% solution of ammonium d-10-camphor sulfonate and place the sample in the sample chamber.

6. Select 'Start' command

7. Confirm that the peak from channel 1 (CD) is 190.4 +/- 1 mdeg at 291 nm. If the peak maximum does not fall within this range, use a small screwdriver to adjust the recessed 'Scale Correction CD' adjustment screw on the back side of the instrument until the ellipticity reads within the appropriate range (See pg 29 in the 'Hardware/Function Manual' for illustrations).

c. Peltier Element

On the occasion that the user is measuring spectra over a wide thermal range, but the temperature changes very slowly or if the user plans to collect spectra at very low temperatures (ie below 0 degrees C) the Peltier element housed within the sample cell holder may need to be replaced. The Peltier element degrades over time and does not conduct heat as efficiently as a new element. If the Peltier element needs replacement, there are two options, the user can remove the sample chamber and send to Jasco for repair, or a Jasco technician can come and replace it. The user cannot do this on their own as the Peltier element must be carefully packed and soldered into place. This is a messy job. The most cost effective plan is to remove the sample cell holder and shipping to Jasco in Easton, MD. To do this, all that must be done is removing the bolts fixing the sample holder in place (ie the entire assembly where the cuvette is placed) which are screwed to the bottom of the sample compartment. The electronics must be

unclipped and the temperature probe removed. This is a rather robust sample chamber, but utmost care must be taken not to scratch or break the sample compartment windows in the light path.

V. Troubleshooting

In addition to the troubleshooting guide provided on pages 31 – 33 in the ‘Hardware/Function Manual,’ this user will offer a few helpful suggestions on dealing with common problems encountered during operation.

a. Peltier changing temperature relatively slowly

The Peltier element may need to be replaced. Slow temperature changes will be noticed especially at very low temperature (ie below 0 degrees C). To prolong the life of the Peltier element and save on costs for replacement/labor, the circulating water bath can be used to help the Peltier achieve these low temperatures. Under normal operation of the CD at room temperature, set the water bath to 18 degree C. For temperatures below 0 degrees C, set the water bath to 14 degrees C. This will make the temperature change more quickly.

b. Amp check error upon initialization

This is an internal test voltage that has no bearing on the analysis or normal operation of the CD. It is advised to contact Jasco upon this occurrence however to discuss symptoms with a technician. It should not be assumed that the instrument can be continually run without further maintenance.

c. HT ‘Too High’ upon initialization

There is most likely a light leak somewhere. The most common place for this is the sample chamber. Make sure the lid to the sample chamber is closed properly and the latch on the far left front of sample compartment is in contact with the sample compartment lid. This latch is

pressed down normally when the lid is closed and controls the shutter being opened and closed when the lid is closed and opened respectively.

Also be sure that there is no sample in the sample holder which could obstruct the light path.

d. HT ‘Too Low’ upon initialization

Alignment of the lamp is likely off. Make sure the protrusion of the light source is not facing either the M0 or M1 mirrors. Gently loosen the fixing screws of the light sources and slightly turn the light source before re-securing. Check again upon initialization to be sure the HT is ‘OK.’

e. Voltage higher than usual with no sample in chamber

The lamp needs to be replaced (**Jasco Part Number 3027**). Regular monitoring at low wavelengths with no sample in the chamber should be performed and recorded routinely to monitor for noise related to the lamp life.

f. Voltage does not increase when sample chamber is closed

Be sure the shutter is open and that the lid to the sample chamber is pressing down on the latch which controls the shutter.

g. High levels of noise

The most likely cause for this problem is high absorption of sample components such as salts. Dilute the buffer concentration and retry. A shorter path length cuvette may also be advisable.

The lamp may need to be replaced (**Jasco Part Number 3027**). See section e above.

The spectral bandwidth may be set too low. Try increasing the bandwidth to allow more light to pass through the sample.

h. Low reproducibility of CD spectra

The most common cause for this problem is slight alterations in sample concentration. It is critical that sample concentrations be maintained in order to obtain consistent and reproducible spectra. It is also critical if the user wishes to compare spectra between proteins with the same number of amino acids.

The lamp may be getting old and may require longer warm up times than 10 – 15 minutes.

The scanning speed may be too high. Lower scanning speeds allow the user to obtain more consistent and reproducible CD spectra. The response time is also longer at slower scanning speeds which allows for a longer averaging time for the signal to become stable.

VI. Contacting Jasco

a. Contact Information and Instrument Serial Numbers

Jasco Incorporated
8649 Commerce Drive
Easton, MD 21601

www.jascoinc.com

Telephone: 800-333-5272
410-822-1220

Fax: 410-822-7526

The current Jasco service engineer is Ron Miller – rmiller@jascoinc.com
(Note, service engineers change with relative consistency)

To contact a service engineer or schedule maintenance, use one of the two telephone numbers provided above.

Jasco J-810 CD Spectropolarimeter: Serial Number B030960750

Jasco Temperature Controller Module: Model PFD-425S, Serial Number A004760835

Jasco Titrator Unit: Model ATS-429S/429L, Serial Number A006060832

Osram 150 W Light Source: Part Number 3027