

Operating Instructions for the JASCO Circular Dichroism (CD) Spectropolarimeter (Model J-1500)

BRWN 3154
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A. General Information

The JASCO J-1500 Spectropolarimeter operates in a fashion very similar to a single beam UV-Vis spectrophotometer. Like any single beam measurement, collection of a reference spectrum is required to correct for sample and instrument artifacts. Like UV-Vis measurements, CD signal strength is directly proportional to concentration (Beer-Lambert Law). However, unlike UV-Vis instrumentation that measures the percent transmittance, the CD signal is the difference in percent transmission of right and left handed circularly polarized light. This difference is on the order of one part in ten thousand of the total absorption. Such small differential measurements are inherently noisy. To compensate for the noise, CD measurements are generally made by scanning slowly and averaging multiple scans.

Due to the different needs of each research group and the variety of cells and sampling techniques, each user is responsible for providing cells/cuvettes and preparative materials. Samples should be prepared prior to arriving to collect spectra.

The software used to operate the instrument is a suite of programs written to perform specific operations. These programs are accessed using the Spectra Manager program. The available data collection and analysis programs are as follows.

Measurement Programs

Spectra Measurement:	Record a CD spectrum at a fixed temperature
Time Course Measurement:	Record a CD signal at a fixed wavelength and temperature as a function of time
Validation:	Used for instrument maintenance
Data Monitor:	Display a CD reading at a fixed wavelength
Variable Temperature:	Record a CD signal at a fixed wavelength as a function of temperature
Temperature Interval:	Record CD spectra at regular temperature intervals
Interval Scan:	Record CD spectra at regular time intervals, at fixed temperature
Temp/Wavelength Scan:	Record CD signal at fixed wavelength at regular temperature intervals

Analysis Programs

Spectra Analysis:	Process simple wavelength and temperature scans
JASCO Canvas:	Manipulate spectral images
Interval Data Analysis:	Process multi-spectra data sets
ORD-E Data Correction:	Used for Optical Rotary Dispersion data
MCD Hysteresis Analysis:	not used

B. Instrument Startup

Starting the instrument and allowing for system warm-up requires approximately 20 minutes and *should not* be rushed. If only data analysis is desired, the spectropolarimeter should not be turned on and operation begun at step 5 below.

1. Sign the logbook and then turn on the Nitrogen purge to remove Oxygen from the system and continue purging until the instrument is turned off. This will prevent oxidation of the optical surfaces from the production of ozone. The Nitrogen flow rate is controlled using the flow gauge on the left side of the instrument and should be set to 40 for 20 minutes prior to turning on the lamp.
2. Check to see that there is no sample or other obstruction of the beam path in the sample compartment and that the lid is tightly closed.
3. Turn on the J-1500 Spectropolarimeter using the power switch located on the left side of the instrument.
4. Turn on the cooling water control valve located on the back of the pillar to the right of the instrument. Check that water is flowing in the tubes inside the sample compartment. Cells must be sealed when operating above 40°C to prevent condensation problems in the sample compartment.
5. Turn on the computer by moving the mouse. Press the <Ctrl> + <Alt> + keys simultaneously and enter your User Name and Password. The Domain entry should be set to ONEPURDUE. Select the correct account number on the purple window that appears.
6. Double click the Spectra Manager icon to start the software.

C. Collecting Spectra

Since the majority of users are interested in a basic wavelength scan, the remainder of this document focuses on use of the Spectra Measurement program. However, the procedures for completing temperature and interval scans are very similar. Contact the person(s) listed at the end of this document if assistance is needed with other programs.

1. Double click on Spectra Measurement in the left column of Spectra Manager. A dialog box counting down the time remaining on the mandatory Nitrogen purge will appear. If the instrument has already been purging for AT LEAST 20 minutes, press the Skip button. The lamp is ignited and instrument initialized.

Note: Skipping the purge timer prematurely will result in serious damage to the instrument!

2. Record the PMT voltage in the logbook. This value serves as an indicator of lamp life.
3. Under the Measurement menu select the Parameters option.
4. Under the General tab, enter the desired scan parameters. Listed below are parameters that can be used as a general starting point. Please note that parameters should be selected and optimized for particular types of samples.

The right column: Start: the wavelength where the scan will begin
 End: the wavelength where the scan will stop
 Data pitch: the data interval; i.e., how often data is collected
 Start mode: select Immediate
 Scanning mode: select Continuous
 Scanning speed: the rate of data collection
 No. of accumulations: used to record multiple scans automatically

The left column: Channels Num: select 2
 Channel 1: select CD
 Channel 2: select HT
 CD Scale: select 200, this is the sensitivity of the instrument
 D.I.T.: select the value that gives $D.I.T \times \text{Scanning speed} < 200$
 Bandwidth: select 1 or 2 nm

5. Use the Cell Unit tab to set a specific temperature.
Warning – When using the cell probe, always press the stop button before removing the external temperature probe from a sample. Failure to do so may result in permanent damage.
6. The Control tab can be skipped. This sets the automatic baseline correction and this function is not used.
7. The Information tab is used to input various sample parameters.
8. The Data tab is used to select where the raw data is stored. Under the Format button select Sample no. Check the box for Send Data to Spectra Analysis.
9. Click the OK button to send the parameters to the instrument.
10. Select Start Measurement under the Measure menu to begin data collection. Be sure the shutter is open.

D. Data Manipulation

Once data collection has completed the data will be automatically transferred to the Spectral Analysis program for processing. This program has only modest image processing and graphing capacities. Typically the data is converted to a text file format (i.e., ASCII) and the data is transferred to a spreadsheet program for further analysis. Use the File → Export option to access the available text formats and drives.

Files may be *temporarily* stored in the D:\User Data for your convenience. Keep in mind that this is a public access machine, hard drives crash, etc. The data may not be there when you return.

E. Instrument Shutdown

If another user will use the instrument within the hour, it may be left on after confirming that the next user will be arriving promptly. Otherwise, follow the procedure below to shut down the instrument.

1. Remove the sample from the sample compartment.
2. Choose File → Exit to close the Measurement program and then choose Program → Exit menu option to close the software.
3. Turn off the J-1500 Spectropolarimeter using the power switch located on the left side of the instrument.
4. Turn off the cooling water supply valve.
5. Turn off the Nitrogen purge.
6. From the Windows menu bar at the bottom of the screen, select Log Off.

F. Miscellaneous

Molar Ellipticity, θ (theta)

The data is normalized by dividing by the path length and concentration. The units of molar ellipticity are (deg·cm²/dmol).

Sampling Considerations

Standard cuvettes used for UV-Vis spectroscopy are satisfactory for most applications. The spectrum of the cell should, however, be measured as some cells will have a CD signature due to residual mechanical stress after annealing.

Protein samples generally have a working concentration range of 0.1 to 1.0 mg/ml with absorbance in the 0.1 to 0.5 range. The total absorbance of the solution being measured should not exceed 1 Abs units.

The sample concentration must be adjusted so that the PMT voltage does not exceed 700 volts at any point in a scan. Data collected at PMT voltages above 700 volts are suspect.

If PMT voltage exceeds 800 volts during a scan, click the Stop button. Operating the PMT above 800 volts stresses the instrument and returns non-linear data.

There is a strong Oxygen absorption band in the UV below 184 nm. Therefore, it is necessary to degas samples when working below 185 nm. This can be done by bubbling Nitrogen through the sample for 10 minutes.

In preparing solutions, use deionized water and phosphate buffers, and avoid the use of chlorides, which absorb strongly below 210 nm. Thus do not adjust pH with HCl.

The instrument baseline will drift with time. When measuring small signals, it may be necessary to “recollect” the baseline about once every hour. An alternative method of drift correction is to subtract a constant to return a KNOWN zero point in the spectrum to zero.

Improving Signal to Noise (S/N)

CD signals are noisy compared to other optical measurements and there are a few things that can be done to improve the S/N ratio.

1. Averaging repetitive scans can minimize Gaussian noise. The noise will decrease by one over the square root of the number of scans. For example: the data obtained from 4 scans has 1/2 the noise of 1 scan while the data from 16 scans has 1/4 the noise.
2. Increasing the response time will also reduce the random noise. However, this requires slowing the scan speed to avoid skewing of the data. In general when setting parameters, the Scan Rate times the Response Time (D.I.T.) should be no greater than 100 for best results. This is also a tradeoff of time for S/N.
3. Increasing the instrument bandwidth can increase S/N by allowing more light into the detector. This is a tradeoff of resolution for S/N.
4. The sample concentration can be increased to give higher readings provided the absorbance remains within range (i.e. PMT voltage not exceeding 800 volts).

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