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BAF_S02_Jasco 1500 Circular Dichroism Spectrometer

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We use this protocol and it's working

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Abstract

This is a general step-by-step to run a sample in the Jasco 1500 CD spectrometer.

Safety warnings

Troubleshooting:

If a spectrum shows nothing, problems could be:

- sample compartment lid not fully closed
- the shutter did not open because you did not set automatic shutter control in the Control tab for setting parameters or did not manually open the shutter.
- the spacer to hold the 1 mm cell in the temperature-controlled cell is upside down preventing any light from reaching the detector.

Before start

Cell holders:

There are two cell holders: Standard & Temperature Controlled

- Standard: no temperature control, holds microsampling cell, holds cylindrical cells and rectangular cells in a frame which adjusts to the cell size.
- Temperature controlled: temperatures of 0 to 100°C, holds only rectangular cells, has two windows which cause a small increase in PMT voltage, Spacer for 1 mm cells, Aviv holders can be used for 1,2,5 mm cells, and there is a stirrer motor that may only be useful with 10mm path length cells.

Sample preparation:

JASCO says that optimal S/N ratio is between 300 and 350 Volts on PMT, absorbance of 1. An absorbance of 2, PMT voltage of 400-450 is acceptable.

In a 1 mm cell, 0.1 mg/ml lysozyme gives an acceptable spectrum. If you do not need a spectrum below 190 nm, you can use higher concentrations of sample.

Buffers:

For list of some buffers to choose or avoid, see table 1.

Acetic acid and acetates should also be avoided for wavelengths below 230 nm.

Chloride ions also absorb at short wavelengths, so PBS cannot be used for short wavelengths: sodium sulfate or fluoride or ammonium sulfate may be suitable for raising ionic strength. DTT and histidine also absorb and should be avoided.

Degassed buffers avoid bubble formation when the sample is heated for melting experiments. Degassing may also reduce oxygen which contributes to absorbance at short wavelengths, and hence reduce signal.

TCEP absorbs but at suitably low concentrations can be used.

One review suggests CHAPS and octylglucoside can be used, but Triton detergents oxidise to UV absorbing material. (J.T. Pelton and L.R. McLean *Anal Biochem*. **277** 167-176, 2000)

Table 01: Cutoff Wavelengths For Common Solvents and Buffers

	A	B
	Solvent System	Cut off (nm) for one mm path length
	Water	<185
	Trifluoroethanol	<185
	Hexafluoroisopropanol	<185
	Acetonitrile	185
	Methanol	195
	Ethanol	196

	A	B
	2-Propanol	196
	Cyclohexane	<185
	Dioxane	232
	(NH4)2SO4 0.15 M	191
	NaCl 0.15 M	196
	NaClO4 0.15 M	<185
	NaNO3 0.15 M	245
	Phosphate 100 mM	<185
	Tris 100 mM	195
	Pipes 100 mM	215
	Mes 100 mM	205
	GdnHCl 4 M	210
	Urea 4 M	210

Running a Sample

1 **Turn on the nitrogen supply**. this will turn on the lamp

1.1 Open both nitrogen cylinders, and open the low-pressure flow valve on the wall mounted manifold. don't change pressure regulators. Both pressure gauges should show a reading and noise during gas flow is normal

1.2 Make sure the flow controller on the left of the instrument is set at 15-20. Typically, this will not need to be adjusted, so checking it should be fine. The system does not warn if nitrogen flow is inadequate, so it is very important that you check this!

1.3 Turn on the instrument, the computer and the spectra manager. once in spectra manager, click on SPECTRA MESUREMENT. The program is set to turn on the lamp after the instrument has purged for 15min. the system is not fully stabilized until the lamp has been on for 45min.

2 Install the holder you need, temperature controlled or standard

2.1 If you are using temperature control, turn on the KOOLANCE circulator by sliding the switch on the box at the back of the instrument. a blue light will come on signifying that it is on.

3 Start SPECTRA MEASUREMENT

4 Go to MEASURE, click on PARAMETERS in the drop down menu or open previous settings from FILE OPEN PARAMETERS. normally it does not matter if the parameters window is in basic or advanced mode

5 In the CONTROL tab of the parameters window, select SHUTTER OPERATED AUTOMATICALLY.

6 In the DATA tab of the parameters window, select AUTOSAVE

7 The software may tell you to scan a baseline. If not, click the blue B icon. Then insert the sample and click the green icon to scan the sample

7.1 If using a spacer for the 1 mm cell, ensure the hole is down

8 Once parameters are set, click the blue 'S' icon to start the scan

9 When finished, use a small transfer pipette to remove most of the liquid. then, shutdown the data acquisition program

10 Turn off the xenon lamp (the yellow bulb icon)

10.1 This may not be necessary but letting the nitrogen flow for 5 minutes before turning off the instrument lets the lamp cool

11 Turn off the nitrogen low pressure flow valve on the manifold and the valves on top of both cylinders

Scanning settings

12 Scan settings are made in Spectra Manager-SPECTRA MESUREMENT which requires turning on the nitrogen supply because the Spectra Measurement program will turn on the lamp. The Spectra Manager manual has descriptions of settings start at p. 130.

13 **Wavelength:**
For proteins 250 to 190 nm is common. If your buffer absorbs, you will not reach 190 nm. Below 185 nm, you need to use a cell with a path length of 0.1 mm to reduce the absorbance of water. For shorter wavelengths, you will have to purge at a nitrogen flow rate higher than normal.

14 **Data Acquisition Interval:**
JASCO suggest 0.2 nm normally. In the CD/ORD manual, they say that the number of data points should not exceed 2001. For UV protein scans, this is not a problem.

15 **Scanning speed:**
This is affected by smoothing time set with D.I.T. data integration time. The software will tell you if the Data Integration Time and scan speed are incompatible. See p. 130 of the Spectra Manager manual.

16 **Numbers of Scans (Accumulations):**
Signal/noise is proportional to the root square of the number of scans. For high sensitivity, JASCO suggests 4 to 50 scans. The absorbance of the sample affects the signal-to-noise ratio. Optimum signal-to-noise ratio is at an absorbance of 0.8686.

	A	B
	Sample absorbance	Numbers of scans to achieve same signal to noise ratio
	1	1
	2	2.5
	3	11

17 **Response or D.I.T.:**

Values within the D.I.T. are averaged to determine the signal at a point. Signal-to-noise is proportion to the root square of the D.I.T.

	A	B
	CD scale mdeg	D.I.T. seconds
	10	0.5 to16
	200	0.125 to 0.5
	2000	0.025 to 0.064

18 **Bandwidth:**

Normally 1 nm. However, wider is suggested for the micro cell. (P. 141 of Spectra Manager manual.) When using the microcell, increased bandwidth (4 nm vs 1 nm) reduced noise above 200 nm. Bandwidths greater than 1 nm may allow scattered light to distort the spectrum.

19 **Scanning at short wavelengths:**

Below 180 nm the absorbance of low concentrations of oxygen becomes significant. JASCO suggests purging with a high flow rate of nitrogen, 80 on the flow meter, to reduce oxygen. 10 minutes of purging can reduce photomultiplier voltage from 1100 to 630 volts. The CD/ORD manual suggests 30 minutes of purging may reduce absorbance a little more. To observe the reduction in voltage, with the sample compartment closed, ensure you have loaded parameters that include monitoring of PMT voltage. set the wavelength to 175 nm using the arrow icon on the toolbar and open the shutter using the icon on the toolbar and watch the HT voltage. However, water also absorbs, so using a 0.1 mm path length cell reduces absorbance. The water may contain oxygen, so degassing solutions may help. JASCO list 175 nm in a 0.1 mm cell as the lower limit for scanning samples in water. Using heavy water, they suggest a lowest wavelength of 171 nm, and 170 nm for trifluoroethanol.

20 **CD scans while raising temperature: melting curves, thermal stability.**

To run a melting curve, use Temperature/Wavelength Scan Measurement Program, or Interval Scan, and view data with Interval Data Analysis Program. In Windows Explorer, you will see .jmb files which are

interval scan files, and jmbv files which are the associated 3D plots. There are two ways to monitor the temperature of the cell, the sensor in the cell holder, and a probe inserted into the cell. JASCO technical support suggests comparing the two. On our instrument, the temperature reading on the holder was about 0,2°C higher than the probe, As expected, temperature measurements in the cell lagged the readings from the cell holder.

Protocol references

SpectraManager_SetupGuide.pdf

There is some training videos at the Jasco website:
https://jascoinc.com/training-video/video-category/spectra-manager-for-cd/