Circular Dichroism Procedure

I. Introduction. This document is a guide for sample preparation, instrument startup, data manipulation, and instrument shutdown. Facility policies are also outlined herein. This document does not discuss methodology or data interpretation. The user should be familiar with Windows operating system and importing files.

The Jasco Model J-810 spectropolarimeter instrument in the Biophysics Core Facility was installed in September of 2000. It is capable of acquiring CD, ORD, and fluorescence spectra. The Spectra Manager Program is used to control the instrument. Within the Spectra Manager program are several options:

1. Spectra Measurement – for data acquisition.
2. Spectra Analysis- for data analysis and conversion.
3. Variable Temperature Measurement – for measuring change in CD with time at a fixed wavelength. Typically used to obtain a temperature melt.
4. Temperature/Wavelength Measurement – for measuring the change in response with temperature. Also, wavelength scans at a constant temperature are obtained. You may obtain spectra at defined temperatures during a melt.

II. Facility Policies.

A. Schedule time at least 48 hours in advance at The Biophysics Core Facility web site at http://biomol.uchsc.edu/webcalendar/. Similarly, you must cancel any user time 48 hours in advance. Failure to do so will result in instrument use charges. If you do not have a login account, you can contact Brooke Hirsch at brooke.hirsch@uchsc.edu to request an account. An account will be set up for you and the information will be emailed to you.

B. Sample cells need to be checked out and returned to the Biophysics Core Facility Manager. If a user breaks or damages a cell, they will be responsible for replacing it. Cells can be ordered from Jasco Instruments, 800-333-5272, www.JascoInc.com.

III. The Instrument and Accessories.

A. Cells and Cell Holders.

1. There are currently 2 cell holders, one with Peltier temperature control and one without temperature control. Cylindrical cells can be used with only the latter of the two cell holders.
2. Peltier cell holder. This holder is ideal for temperature melts. Only rectangular cells can be used with this holder. Samples are not recoverable if a rectangular cell less than 1 mm path length is used. There is a spacer that should be used with narrow cells. This is a black piece of metal with a hole in it. Place the hole near the bottom so that the light beam can pass through it. The spacer may also be used to determine minimum volume required. Ensure that the meniscus of the sample is not below the hole. For 1mm cells, the volume used is usually 200 μL.
3. Holder for cylindrical cells. The cylindrical cells are designed for larger sample volume and sample recovery. No variable temperature control is available with these cell holders.

B. Care and Cleaning Of the Sample Cell.

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1. Routine cleaning should be performed with the cell cleaner located next to the instrument. Turn on the vacuum. Place the cell upside down over the cell washer. Squirt water/soap/or ethanol into the adjacent compartment to wash. The standard procedure is to rinse 3 times with water and 3 times with ethanol. To dry the cell, stream nitrogen gas from the tank into the cell.
2. For stubborn dirt or proteins, you may use the CD cleaning solution of chromic-sulfuric acid cleaning solution. Do NOT use alkali, hydrofluoric acid, or phosphoric acid on the quartz cell.
3. Avoid scratching the cell. After cleaning, avoid touching the sample cell. Fingerprints can cause artifacts in the data. A kimwipe can be used to remove any fingerprints or material from the cell.

IV. Sample.

A. Concentration. The following parameters are a starting point for sample concentrations.
    1. Far-UV CD Spectrum. 1-mm rectangular cell. 0.1 mg/ml solution, ~ 0.2 ml. Set wavelength to 180 – 260 nm. Ensure that the baseline from about 240 – 260 is flat and zero after subtracting the blank.
    2. Near-UV CD Spectrum. 1-mm rectangular cell. 0.1 mg/ml solution, ~0.2 ml. Set wavelength to 200-350 nm.

B. Centrifuge or filter the sample to remove aggregates. A UV spectrum can help to determine whether aggregates are present.

C. Obtain the exact concentration the sample. An exact concentration for CD analysis is required. Also, published data must be normalized so that comparisons among different samples can be done. Suggestions to determine concentration: Amino acid analysis, a UV spectrum can be used if the exact extinction coefficient is known.

V. Buffer.

A. Test the following to ensure that the buffer solution is okay: Solubility, transparency in the region of interest, optical activity, stability during the experiment, solvent polarity, *i.e.*, the interactions of the solvent with the analyte.

B. Use UV-grade solvents and buffers.

C. Avoid using H_2O that had been stored in a polyethlyene bottle for a long time. The polymer additives may elute resulting in the water losing its transparency.

D. Jasco recommends flowing the solvent through an activated alumina (neutral) column before use.

VI. Instrument Startup.

A. Log onto the computer. Each lab has a log on and password. Your instrument time will be tracked by your logins.

B. Turn on N_2 gas flow at the tank or the wall. The flow of the meter ball should be at or above 40. This gauge is located on the left of the instrument. It takes about 5 minutes to purge the compartments.
   NOTE: The Xe lamp will generate ozone which is harmful. The N_2 gas prevents the formation of ozone. Ozone deteriorates the optics.
   NOTE: Ozone absorbs at 200-300 nm and 600-700 nm. Ensure that N_2 level is adequate for your run.

C. Turn on the power to the CD at the lower left of the front of the instrument.
D. Ensure that the silver selection knob at the right of the instrument is set to "CD".
E. If the Peltier temperature controller will be used, turn on the water bath that is set at 20°C and the Peltier control device on the bench.
F. Start the Spectra Manager Program. There is only 1 instrument attached. Your choice of experimental measurements will be as follows:
    1. Spectrum Measurement to acquire a CD spectrum.
    2. Time Course Measurement.
    3. Interval Scan Measurement.
    4. Data Monitor – Not applicable.
    5. Environment – Not applicable.
    6. Variable Temperature. Measures the change in CD with time at a fixed wavelength while the temperature changes. This is the typical program to obtain a melt.
    7. Temperature/Wavelength. To obtain a temperature melt and spectra at designated temperatures.

G. Select an experimental measurement. At this time, a self-diagnosis will be performed. Wait until it is completed to proceed. A box will appear with the message, "HT value too small". This is because you have not turned on the lamp. Select "Ignore" and continue.
H. Once the main window has opened, go to "Control" then "Light source". Check the box next to the lamp and click apply. This will turn the lamp on. Confirm the lamp is on by the green indicator light on the instrument. The lamp should warm up for a minimum of 5 minutes before use.
I. To turn on the temperature control device. Go to "Measurement", then "Accessory". Select Jasco Peltier Device (the only option) and click apply. Next, go to "Control", then "Accessory" and set the temperature. Click apply and close the window. After setting the temperature, you have to push "Start" on the actual temperature control device on the bench. The top reading is the set temperature. The bottom reading is the actual temperature.

VII. Data Acquisition.

A. CD Spectrum. To acquire a CD spectrum, "Spectrum Measurement" was selected above.
    1. Select "Measurement", then "Parameters" to set the parameters for your experiment. Select the "Parameters" tab. The following are only a guideline for peptide/protein solutions.
    *Sensitivity: Standard (100mdeg)
    *Start: 250 (for far-UV readings) Max is 800
    *End: 185 (for far-UV readings) Min is 170
    *Data pitch: 0.5nm (amount of data points, here it will give a data point every 0.5nm)
    *Mode: Continuous
    *Scanning Speed: 50 nm/min
    *Response: 2 sec
    *Bandwidth: 1 nm
    *Accumulation: 6

2. Select the “Data Mode” tab, nothing should be changed. The following are the settings:
   * Num. of Channels: 2
   *Channel 1: CD
   *Channel 2: HT
3. Select the “Data File” tab. Select autosave to save files as they are acquired and specify a file name. In the directory tab, select a folder where the files will be saved.
4. Select the “Option” tab to enter sample specific information for your records.
5. Click [OK] to apply the parameters and close the dialog box.
6. If the Peltier device is in use, make sure you chose the temperature control accessory and set the temperature (see above).
7. Click [Start] to begin data acquisition.

VIII. Data Processing. After the instrument has completed acquiring data, the data will be displayed in the Spectral Analysis program. The following tasks will be done from Spectral Analysis.

A. Spectral Data. If autosave was not enabled, then save the data under “File”, then select “Save”. This data file will contain mdeg and HT.
B. Correct the analyte data by subtracting the blank. There is more than one way to perform a blank subtraction. Both the data mode and interval of each spectrum must be the same. Overlay the two spectra, one of the blank and one of the sample. This can be done by opening both files and dragging one spectrum into the window with the other spectrum. Or by selecting “File”, then “Overlay”. Next select “Processing”, then “Subtraction”. The top line in the window shows the order of subtraction. If incorrect, press [Exchange]. Press [Apply]. Save the results under “File”, then “Save”.
C. To save data as a text file simply go to “File”, then “Save as”. Make sure ASCII (*.txt) is selected. Type your file name and click save.
D. Convert from CD mdeg to mean residue ellipticity [θ]. You will need the exact concentration for this step. Determine Mean Residue Molar Concentration, C_r, according to one of the following equations where n is the number of residues per molecule.
   (1) $C_r = n * C' / M_p$ where C’ = concentration in mg/ml and M_p is the molecular weight of the molecule.
   (2) $C_r = n * C_p$ where C_p = molar concentration, M.
   Select “Processing”, then “CD Options”, then “Optical Constant”. Next, Select [Molecular] to determine molecular circular dichroism absorption. The Specified Channel will be “Ch. 1 CD (mdeg)”. Ignore channel 2. The Calculation Mode will be “Molecular Ellipticity”. Enter the correct cell length & concentration. The “Molecular Weight” and “Magnetic Field” boxes can be left blank. Press [OK] and the resulting spectra will be displayed. Save this under “File” then “Save”. For “Molar Ellipticity”, select “Specific”, enter cell length and concentration as well as molecular weight. Click [OK] and the resulting spectra will be displayed.
E. Print the resulting spectra with “File” then “Print”. To print the CD only spectrum. Select “Processing” then “Peak Processing” then “Peak Find”. Increase noise level to 100000 so that no peaks will be found. Click on [Execute]. If peaks are found, click on the peak in the upper window then select [Delete]. Do this to remove each peak otherwise they will be printed. Click on [Print]. Click on [OK] to close the window.

F. ASCII table of x, y data. Select "Processing" then "Common Options" then "Data Dump". Next to "Dump", enter the wavelength range. Thin Out the number of data points that you want to remove. If you acquire data every 0.1 nm and want to save data every 1 nm, then enter "9". Click [OK]. A window containing text data appears. Click on [Copy] to copy the contents to the clipboard. Click on [OK] to close the Data Dump window. Open a text editor such as Notepad, Wordpad, or Word. Press Ctrl-V to copy the contents into the program. Save the data as a text file. Print the data from the text editor.

IX. Instrument Shutdown.

A. Turn off the lamp by going to "Control" then "Light Source". Uncheck the box next to the lamp and select apply. Make sure the lamp indicator on the instrument is no longer illuminated. If in use, turn off the water bath and the temperature control device. Turn off the CD.

NOTE: Do not have the water bath on when the lamp is off. Condensate may accumulate on and destroy the lamp and optics.

B. Exit Spectra Manager and Spectrum Analysis programs.
C. Log out of the computer and turn off the computer if there are no other users scheduled after you.
D. Let the nitrogen gas run for about 5 minutes after you have turned off the lamp. Turn off the N_2.
E. Report any problems to the facility manager.

X. References.

A. Jasco Instruments, http://www.JascoInc.com.
B. CD Tutorials: http://www.med.unc.edu/wrkunits/2depts/biochem/MACINFAC/cd.html
http://www.ruppweb.org/cd/cdtutorial.htm
http://www.ap-lab.com/circular_dichroism.htm