CD or ORD-E Temperature Melt Procedure

I. Introduction. This document is a guide for obtaining temperature melts with the CD instrument. This document does not discuss methodology or data interpretation. The user should have been trained on the *Circular Dichroism Operating Procedure*. Refer to that document for precautions and other information.

II. 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" or "ORD".

E. Turn on the Peltier temperature controller by turning on the water bath which is set at 20°C and the Peltier control device on the bench.

F. Start the Spectra Manager Program.

G. Select "Variable Temperature Measurement".

H. Select the temperature control device by going to "Measurement" then "Accessory". Slelct the Jasco Peltier Device and click apply. Next to to "Control", then "Accessory" and set the temperature to your starting melt temperature. Click apply then close the box. Finally, press "Start" on the actual temperature control device.

I. Select "Measurement" then "Parameters" to set up your acquisition parameters.

1. Select the "Parameters" tab to set the parameters for your experiment. The following are a guideline for peptide/protein solutions using a 1mm cell.
*Wavelength: 222nm
*Start: 4
*End: 80
*Data Pitch: 1
*Delay time: 30 seconds (This is the time at the beginning after the start temperature is reached and before the experiment commences)
*Temperature Slope: 50 C/hr (can also set in C/min)
*Return to start temperature: select this option
*Sensitivity: standard (100mdeg)
*Response: 2 sec
*Bandwidth: 1nm
2. In the Data Mode tab, ensure that the correct mode is selected.
*Number of Channels: 2
*Channel 1: CD or ORDE
*Channel 2: HT

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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.

J. If you plan to acquire fluorescence data, please refer to the *CD/ORD-E & Fluorescence Acquisition Procedure*.

III. Data Acquisition.

A. Press the [Start] button.
B. At the end, Spectrum Analysis will have your temperature melt spectra. If you did not use auto-save feature, save all data.

IV. 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. This can be used to process (***e.g.***, subtract the buffer, perform a data dump, ***etc***.) and save the corrected spectra as usual. Print the spectra. To overlay spectra, drag them all into one window.
B. Temperature Melt Data. Print the data. Perform a data dump as for a wavelength spectrum except do not strip out any data points. Save and print the data.
C. Wrap Up. Create ASCII table of x, y data and print it. Save the data as ACSII (*.txt). You will need a fitting algorithm and program for the temperature melt data.

V. 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.