User Guidelines & Standard Operating Procedure
for the

AVIV Circular Dichroism Spectrometer

1. Theoretical Background

Circular Dichroism is a form of spectroscopy, similar to ultraviolet or fluorescence spectrophotometry, however CD employs circularly polarized light rather than linearly polarized or unpolarized light. Circularly polarized light is composed of both left and right electromagnetic waves, as explained in greater detail below. The right and left components of circularly polarized light are absorbed differently by a given sample, and can be used to deduce various structural features. The differential absorption of radiation polarized in two directions as function of frequency is called dichroism.

Electromagnetic radiation is composed of electric and magnetic waves, oscillating perpendicular to each other and perpendicular to the direction of propagation. In the case of unpolarized light, the electric component can oscillate in all directions perpendicular to the magnetic component and the direction of propagation. In linearly polarized light, the electric component only oscillates in a single plane perpendicular to the direction of propagation [see Figure 1-1(a)]. In circularly polarized light, the magnitude of the wave is constant, but the direction oscillates such that the wave travels in a helix in the direction of propagation (Figure 1-1(b)]. Circular polarization may be referred to as right or left, depending on the direction in which the electric field vector rotates (Rodger and Norden, 1997).

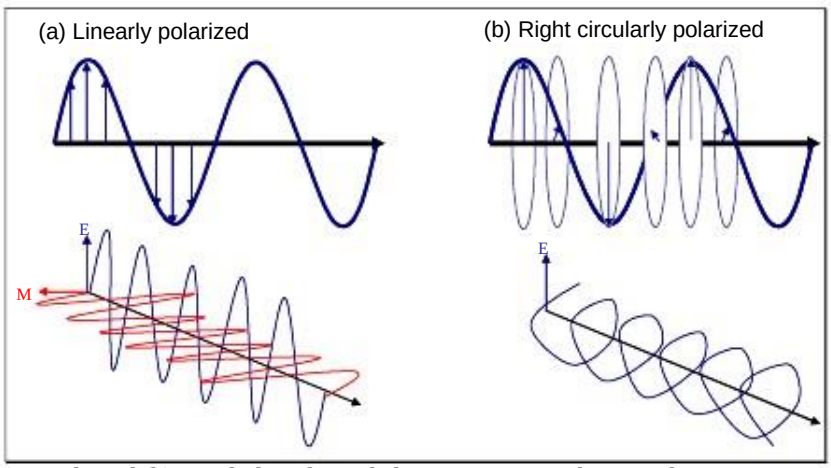


Figure 1-1: (a) Linearly and (b) circularly polarized electromagnetic radiation. The blue arrows illustrate the direction of the electric field (E). The magnetic field is represented by (M) and is always perpendicular to E. Note: M is not included in (b). (Figure adapted from Rodger and Norden, 1997).

Circularly polarized light used in CD experiments is composed of both left and right electric components. When circularly polarized light passes through an asymmetric chromophore (chiral molecule), or a symmetric chromophore in an asymmetric environment, it is changed in two respects. First of all, the magnitudes of the counter-rotating electric components become unequal because the molar absoptivity ( ) of right and left polarized light are unequal. This difference in absorption of right and left polarized light is measured by the CD spectrometer as a function of wavelength. Secondly, the direction of the electric vector no longer trances a circle, instead it traces an ellipse, and the major axis of the ellipse rotates due to differences in refractive indices (Berndt, 1996). See http://www.enzim.hu/~szia/cddemo/edemo0.htm for a very useful demonstration of the affects of absorption on circularly polarized light.

The differential absorption of left vs. right circularly polarized light imparts a variety of structural and chemical information. In general, CD can be used to determine the optical isomerism and secondary structure of molecules. Like other forms of absorption spectroscopy (ultraviolet-visible, infrared), CD spectroscopy is particularly powerful in monitoring conformational changes. In the region of 230-178 nm, CD spectra can be used to deduce backbone conformational changes while CD effects at longer wavelengths (>230 nm) isolate aromatic chromophore contributions and reflect more global, three-dimensional properties of the protein (Berndt, 1996; Rodger and Norden, 1997).

CD spectroscopy has been extensively applied to protein and DNA conformational studies, but care must be taken not to over-interpret the results. Various studies have estimated secondary structural content (α helix, parallel and antiparallel sheet, or random coil), or investigated conformational changes induced by monomer-oligomer interactions, substrate binding, or protein denaturation. Aromatic residues, if unusually abundant, can have significant effects on the CD spectra in the region < 230 nm complicating analysis (Berndt, 1996). Modern secondary structure determination by CD are reported to achieve accuracies of 0.97 for helices, 0.75 for beta sheet, 0.50 for turns, and 0.89 for other structure types (Manavalan & Johnson, 1987).
CD spectra can be recorded vs. temperature to observe thermal denaturation, or vs. time for kinetic data.

The conditions under which the CD spectra are measured can significantly affect the results and should be carefully controlled. See Table 1-1 for some suggested conditions.

The CD signal generated by a sample has been reported in a number of different ways in the literature. The most commonly used units are mean residue ellipticity (degree cm^2 $dmol^{-1}$) and the molar circular dichroism or delta epsilon (liter mol^{-1} cm^{-1}) (Berndt, 1996).

For additional information on CD theory and measurement, see Woody (1985), Greenfield (2004) or any of the reviews by experimental CD spectroscopist W. Curtis Johnson (e.g., Johnson, 1988; Johnson, 1990; Johnson, 1992).

Table 1-1: Suggested parameters for recording CD spectra (Berndt, 1996)

Parameter	Suggested Conditions
Peptide Concentration	Total absorbance of the cell, buffer, and protein should be between 0.4 and 1.0 abs (theoretically, 0.87 abs is optimal).
Measurement Time	To achieve adequate signal-to-noise (S/N is proportional to the square-root of the amount of time measuring the spectrum), a spectra for secondary structure determination (260-178 nm) should be recorded for 30-60 minutes (plus an equivalent amount of time for a baseline).
Environmental Considerations	Oxygen absorbs strongly below about 200 nm so very extensive purging with pure (oxygen-free) nitrogen (>16 liters/min) is necessary for these measurements.
Buffer Selection	A typical buffer used in CD experiments is 10mM phosphate, although low concentrations of Tris, perchlorate or borate are also acceptable. Potassium fluoride is preferred to NaCl for increasing the ionic strength as the chloride ion has a strong UV absorbance at low wavelengths.
Solvent Selection	The CD spectra of peptides have been reported in a number of solvent systems, for example: Trifluoroethanol (TFE) and hexafluoroisopropanol (HFIP) may promote helix conformations, and may mimic an in vivo system (e.g.; Waterhouse & Johnson, 1994).

1.4 Instrumentation

The CD spectrometer can be used to conduct wavelength, temperature, kinetic, and titration experiments. CD spectra can be recorded as a function of wavelength, time, temperature, pH, or concentration.

The instrument includes software by AVIV for instrument control, data acquisition, and processing in a Windows environment. All experimental parameters are accessed through pull down menus. Some of the specifications of the CD spectrometer are listed in Table 1-2.

Table 1-2: Aviv CD spectrometer specifications

Parameter	Specification
Wavelength Range	170 nm to 875 nm
Stray Light	Less than 0.001% at 200 nm, less than 0.1% over operating limits.
Slit Program	Constant bandwidth from 0.01 to 12 nm. Automatic slit closure option to protect sample and optics from UV light.
Baseline Drift	Less than +/- 0.1 mdeg per hour after a 30 minute warm-up, providing environment is held at 24°C +/- 2°C.
Light Source	150 Watt suprasil Xenon lamp controlled by a high stability, constant current, DC power supply.
RMS Noise	With a 1.0 nm bandwidth, no sample, and a 4 second time constant, RMS noise is less than: 0.1 mdeg at 185 nm 0.08 mdeg at 200 nm 0.06 mdeg at 500 nm

The CD spectrometer can be divided up into 7 main components (See Figure 1-2):

1) Light source compartment:
The light source is a 450W Xenon arc lamp with a synthetic fused silica (Suprasil) envelope. The light from the lamp is focused onto the monochrometer entrance slits by an ellipsoidal mirror and a flat mirror. Cooling coils in the lamp house dissipate the heat generated by the lamp.

2) Monochrometer:
The light produced by the lamp is dispersed by the monochrometer via a series of slits, mirrors, and prisms. The monochrometer is a "w" double design which means that it contains two fused silica prisms in series. This design produces better wavelength resolution and minimizes stray light.

The entrance and exit slits are moved synchronously using an external drive. The width of the slit openings are controlled by a stepping motor, and determine the wavelength range sent to the sample. The maximum slit opening of 3.6 mm corresponds to different spectral bandwidths at different wavelengths. However; if the constant-bandwidth mode is used, the program converts the desired spectral bandwidth (in nm) into a slit width (in mm).

An external drive moves the prisms to select for a given wavelength, starting with longer wavelengths and moving to shorter wavelengths. The step size of the external drive corresponds to a 0.05 nm change in wavelength, and this sets the limit to which wavelengths can be selected.

3) Polarizer compartment and modulator:
An achromatic lens within the polarizer focuses the light in the same location in the sample space, regardless of wavelength. The light is linearly polarized by an MgF_2 polarizer, and then passes through a photoelastic modulator. The photoelastic modulator alternately produces $+90^o$ and -90^o retardation via a 50 kHz oscillator, and therefore produces left and right circularly polarized light.

4) Sample compartment:
Circularly polarized light passes through the sample in this compartment. The compartment can contain a variety of accessories such as a temperature control accessory.

5) Detector compartment:

Light is detected by a high speed, high intensity photomultiplier tube (PMT). The PMT is normally operated in constant current mode, and the applied dynode voltage is varied as necessary to maintain a constant PMT current as the light energy changes. The tube output is converted to a voltage by a preamplifier, and sent to the electronics.

6) Electronics:

The electronics are composed of specialized CD signal processing circuits and general control electronics.

7) Computer system:

The computer that is connected to the CD spectrometer is a Pentium II CPU. The software communicates with the instrument and accessories via a serial port.

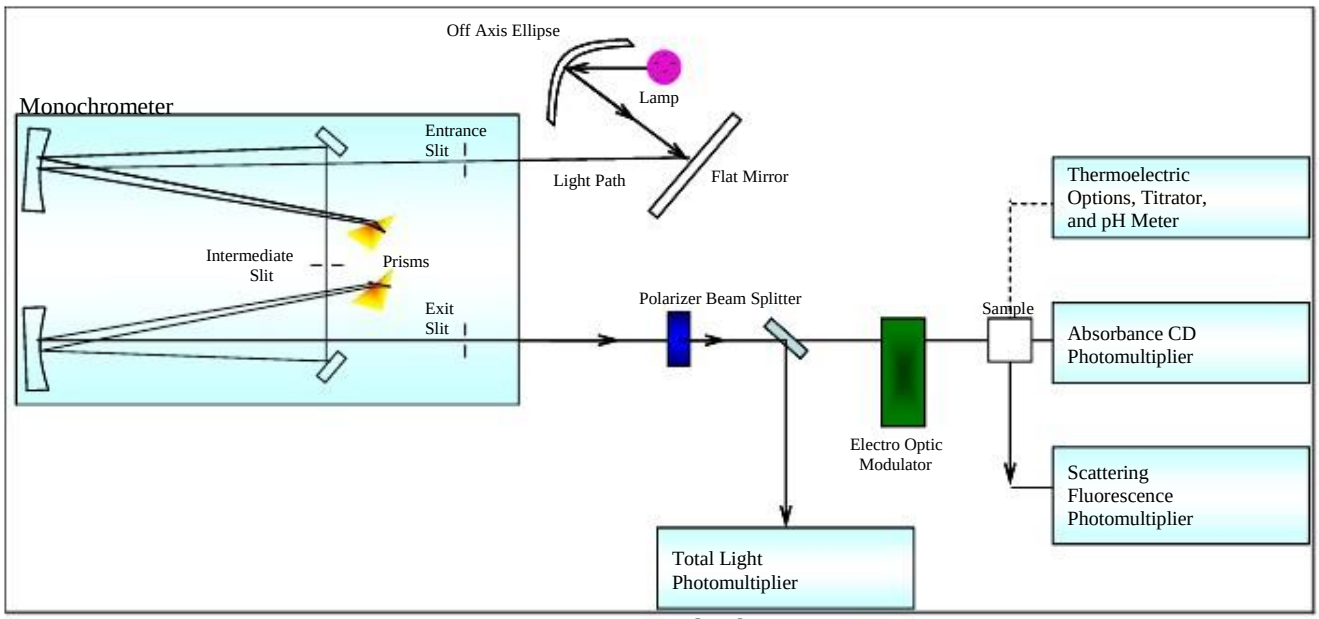


Figure 1-2: CD spectrometer Optical Schematic
(Adapted from the Aviv Model 202 Manual).

1.4.1Instrument Accessories

1.4.1.1 pH titrator

The pH measurement system includes a pH meter and micro-probe electrode. A modified cuvette cap and electrode mount for 10 mm cell are used for sample analysis. The pH can be tested from 0 to 14 pH. Use of the pH titrator requires the Automated Syringe Pump System.

1.4.1.2 Syringe pump

The automated syringe pump system is used for experiments involving titrations, and includes two 500 µl syringes. The syringe pump operates under data system control.

1.4.1.3 Cell adaptor

There are cell adapters available that allow you to use 1 mm, 2 mm, and 5 mm cells in the 10 mm cell sample holder.

2. POTENTIAL HAZARDS

2.1 UV Hazard

Hazardous UV radiation is emitted by the UV lamp in the instrument. This radiation can cause serious damage to your eyes. NEVER look directly at the UV source lamp; the lid should always be shut during data acquisition. if the lamp is operating and the sample compartment must be open; wear safety glasses manufactured to an approved standard and which are certified or otherwise warranted to protect your eyes from UV radiation.

2.2 Chemical Hazards

Use of the Aviv CD system and accessories may involve materials, solvents and solutions which are flammable, corrosive, toxic or otherwise hazardous. Careless, improper, or unskilled use of such materials can create explosion hazards, fire hazards, health hazards, and cause damage to equipment and property.

The spectrometer is connected to a liquid nitrogen cylinder. When it reaches room temperature, liquid nitrogen expands to a gas volume of over 600x the volume of the liquid, and can quickly displace oxygen. As a result, a leaking liquid nitrogen tank presents an asphyxiation risk, and any leaks (beyond the regular venting of the cylinder) should be reported immediately to the Instrumentation Technician, and the room evacuated.

The spectrometer is also connected to a Merlin Chiller. This water bath contains a small amount of chloramine-T, which is hazardous. Contact the Instrumentation Technician if the solution in the water bath is low or looks dirty.

3. PERSONAL PROTECTIVE EQUIPMENT

The sample to be analyzed may contain potentially infectious or hazardous material, so standard laboratory protective equipment must be worn (latex or nitrile gloves, approved safety glasses, lab coat). Closed-toe and heel footwear constructed of resistant material is also required for all laboratory activities.

See the WLU Laboratory Health and Safety Manual for additional information on personal protective equipment: http://www.wlu.ca/documents/23120/Laboratory_Health_%26_Safety_Manual__Feb_2007_Final.pdf.

4. SPILL AND ACCIDENT PROCEDURES

4.1 Accidents

All incidents must be reported to the Instrumentation Technician and if applicable, a student's supervisor.

The Instrumentation Technician will insure that all accidents, incidents and near misses are reported to the Environmental/Occupational Health and Safety (EOHS) Office via the WLU Employee Accident/Incident/Occupational Disease Report form (www.wlu.ca/eohs/forms). To meet regulatory requirements, these forms must be submitted to EOHS within 24 hours of occurrence, with the exception of critical injuries, which must be reported immediately to the EOHS Office by telephone. Critical injuries include any of the following; place life in jeopardy, produce unconsciousness, result in substantial loss of blood, involve fracture of a leg or arm but not a finger or toe, involve amputation of a leg, arm, hand or foot, but not a finger or toe, consist of burns to a major portion of the body, or cause the loss of sight in an eye.

Additional details regarding incident reporting can be found in the WLU Accident Incident Procedure (www.wlu.ca/eohs).

4.2 Spills

4.2.1 Spills Inside the CD Spectrometer

Before using ANY hazardous materials, make sure you understand the proper clean-up procedure. Contact the Instrumentation Technician for specific instructions for spills inside the sample compartment. Any spills in the sample compartment should be wiped up immediately The exterior surfaces of the CD spectrometer should also be kept clean.

1. Review the MSDS, if not done so before commencing the experiment, to determine the protective equipment, spill cleanup, and disposal protocols that are necessary.
2. Wear appropriate personal protective equipment, and contain the spilled material first using an appropriate spill kit.
3. Report the spill to the Instrumentation Technician, who will advise the user on the best way to clean up the spill.
4. Record the spill and cleanup procedure in the CD log book.

4.2.2 Spills Outside the CD Spectrometer

The WLU Laboratory Health and Safety Manual provides detailed instructions for dealing with major and minor spills. Do not attempt to clean up a spill if you have not been properly trained, or if you are unsure of the proper procedures. Before using ANY hazardous materials, make sure you understand the proper clean-up procedure. The Environmental/Occupational Health and Safety Office is also available to provide guidance at ext. 2874. The guidelines below are summarized from the WLU Laboratory Health and Safety Manual.

Determine if the spill is a major or minor spill (see Table 4-1).

1. For major spills:
   a. Evacuate the lab, close the doors, restrict the area, and notify others in the area of spill, including your supervisor and the Instrumentation Technician if possible.
   b. Call ext 3333 (Community Safety and Security).
   c. Activate the fire alarm if there is risk to the safety of other people in the building.
   d. Be available to provide technical information to emergency responders.
2. For minor spills:
   a. Attend to injured or contaminated personnel.
   b. Restrict the area and notify others in the lab of the spill, including your supervisor and the Instrumentation Technician if possible.
   c. Take action to minimize the extent of the spill.
   d. If flammable material is involved, turn of ignition sources (power, Bunsen burners).
   e. It is the responsibility of the user of the hazardous material to clean up the spill if he/she feels it is safe to do so.

f. Select and wear all appropriate personal protective equipment.
g. Apply spill pillow/pads or other absorbent material, first around the outside of the spill, encircling the material, then absorb to the center of the spill.
h. Dispose of all materials used to clean up the spill in a sealed container.
i. All personal protective equipment must be disposed of correctly, and must not be worn outside the laboratory.
j. Label and dispose of all bags or containers as hazardous waste.

3. For chemical spills on the body:
   a. Remove all contaminated clothing.
   b. Flood exposed area with running water from a safety shower for at least 15 minutes.
   c. Have another individual contact 9-911 and ext 3333 to obtain medical attention.
4. For chemicals splashed in the eye(s):
   a. Immediately rinse eyeball and inner surface of eyelid with water continuously for 15 minutes. Forcibly hold eye lid(s) open to ensure effective wash behind eyelids.
   b. Have another individual contact 9-911 and ext 3333 to obtain medical attention.
5. IN ALL CASES: Report the incident to your supervisor and the Instrumentation Technician.

Table 4-1: Guidelines for classification of a major spill

Material	Quantity
Air and water reactive materials	All quantities
Flammable liquids	Greater than 4L
Combustible liquids	Greater than 4L
Non-flammable organic liquids	Greater than 4L
Concentrated acids	Liquids greater than 1L Solids greater than 1kg
	Liquids greater than 1L Solids greater than 1kg
Concentrated bases and alkalis	Greater than 30 ml Liquids greater than 1L Solids greater than 500g
Mercury	Liquids greater than 100 ml
Oxidizers	Solids greater than 50g At the discretion of laboratory personnel
Highly toxic, highly malodorous material	If the leak cannot be stopped by closing the valve on the gas cylinder
Low hazard material	
Compressed gas leaks	

5. WASTE DISPOSAL PROCEDURES

If any hazardous chemicals are used for sample analysis or preparation, they must be disposed of properly, as outlined in the WLU Laboratory Health and Safety Manual.

6. PROTOCOL

Anyone using the CD spectrometer must receive hands on training. This document is a summary of the procedure and is only intended to help you remember the various steps.

6.1 Instrument Sign-up

All users must fill in the Instrument Sign-Up sheet and inform the Instrumentation Technician at least 24 hours before using the instrument.

If you are conducting a particularly sensitive measurement, the instrument can be calibrated immediately before your analysis. Contact the Instrumentation Technician for calibration.

6.2 Cuvette Cleaning

Cuvettes are supplied by each individual/lab using the spectrophotometer, and users are responsible for ensuring that cuvettes are properly cleaned and stored. There are different types of cuvettes with different path lengths and volumes. Use the appropriate adaptor for each type of cuvette.

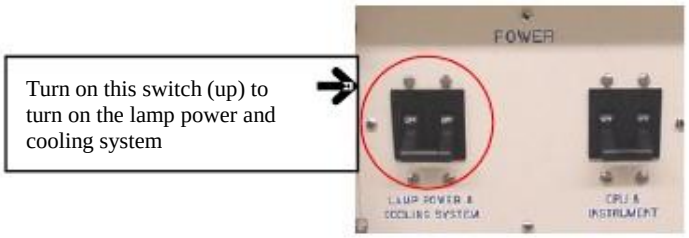
1. Cuvettes must be made of quartz.
2. Avoid handling the cells by the polished surfaces.
3. Dirty cells are the greatest single source of error in spectrophotometry.
   Common contaminants on cuvette surfaces include:
   a. Synthetic detergent solutions or stop cock grease;
   b. Growth of micro-organisms in buffer or reagent solutions which will affect blank values by both their fluorescence and light scattering properties;
   c. Filter paper which leaves phenol residues from the original wood.
4. Cuvettes should be washed in chromic acid or potassium hydroxide in ethanol overnight to be completely clean. Proteins can be removed using equal parts 3N HCl and 50% ethanol. Do not use acetone. Do not clean with hydrofluoric acid which will attack quartz, do not leave highly alkaline solutions in a cuvette for an extended period of time.
   a. Dr. Jelokhani-Niaraki has a non-toxic peroxide based cleaning agent that can also be used if a cuvette is exceptionally dirty (ask the Instrumentation Technician if you would like to use this solution).

5. Solution spillage should never be allowed to dry on the cells and should be wiped off with a kimwipe or lint free cloth.
6. Rinse the cuvette with distilled water followed by methanol, and dry with a stream of nitrogen (do not wipe with kimwipes as this can scratch the cuvette).
7. The cleanliness of the cuvette can be tested by comparing the instrument baseline to the baseline when the empty cuvette is in the cell holder. The deviation should be less then 5 millidegrees and the cuvette baseline should not have any positive or negative peaks.

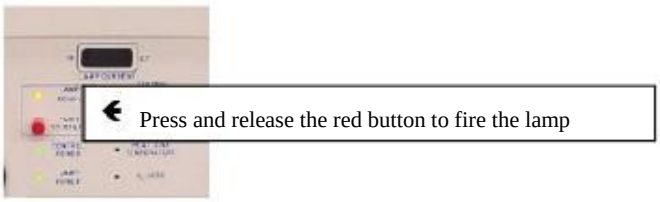
6.3 Starting up the CD Spectrometer

1. Check the level of nitrogen in the cylinder and make sure that the cylinder is not close to empty.
   a. A FULL large cylinder of liquid nitrogen will last for approximately 20 hours; a small cylinder for 6 hours.
   b. Note: some of the gauges are unreliable and therefore the user must pay attention to the nitrogen levels throughout the experiment. If the nitrogen runs out during an experiment it will PERMANENTLY DAMAGE the instrument. If it is running low (dropping below the red lines on the flow meters, or the red line on the cylinder regulator) IMMEDIATELY call the Instrumentation Technician. If the Technician or Dr. Jelokhani-Niaraki cannot be reached immediately, stop the experiment and proceed to shutdown the CD spec properly as outlined in section 6.8.
2. Turn on the nitrogen gas using the main valve and turn the pressure regulator to the red line (35 psi). If you will be conducting an experiment in the far ultraviolet (below 200 nm) the nitrogen flow has to be adjusted. Please contact the Instrumentation Technician if this is the case.
3. Wait approximately 20 minutes while the instrument is purged with nitrogen gas (oxygen in the system will destroy the lamp and optics).
   a. Meanwhile, check the gas flow meters to make sure all are above the red lines:
      - The lamp flowmeter should be above 12.
      - The monochrometer flowmeter should be above 20.
      - The polarizer flowmeter should be above 6.
      - The sample flowmeter should be above 8.
4. Check the water in the Merlin cooling unit to make sure it is full and free of microbial growth.
   a. If algae growth is apparent, contact the Instrumentation Technician before proceeding with the experiment.

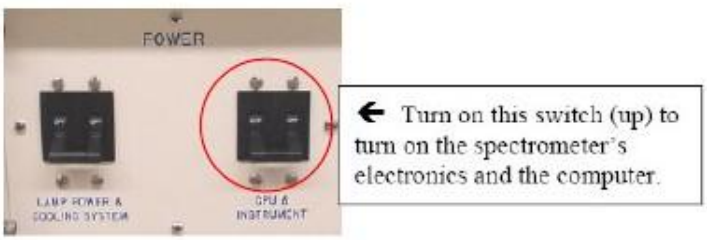
5. When 20 minutes have passed, turn on the "Lamp power and cooling system" using the power switch below the spectrometer. Then turn the chiller on. NOTE: the computer and instrument switches should be OFF at this point.



6. Wait approximately 30 seconds for the yellow lamp ready light to go on.
7. Fire the lamp by pressing and releasing the RED Start button (below the lamp ready light). Do not hold the button down.



8. Make sure that the lamp is ignited and drawing a current.
   a. You should see the red LED's in the "Lamp Current" meter. If they have not lighted, then the lamp did not arc on. Wait a few seconds until the yellow "lamp ready" is on again, and push the RED Start button again. If the lamp does not light after 3 or 4 tries, stop and contact the Instrumentation Technician.
9. Record the lamp hours in the log book (hours found on the LED display located below the main instrument).
10. Turn on the instrument switch and the CPU using the power switch below the spectrometer.



11. Start the software using the CD-215 icon on the desktop.
    a. There is no username or password.
    b. Ignore the warning concerning the pH meter.
12. Let the instrument lamp warm up and equilibrate for 30 to 40 minutes before proceeding with measurements.
    a. During this time the sample can be prepared (section 6.4), and the method set up on the computer (section 6.5, steps 1-2).

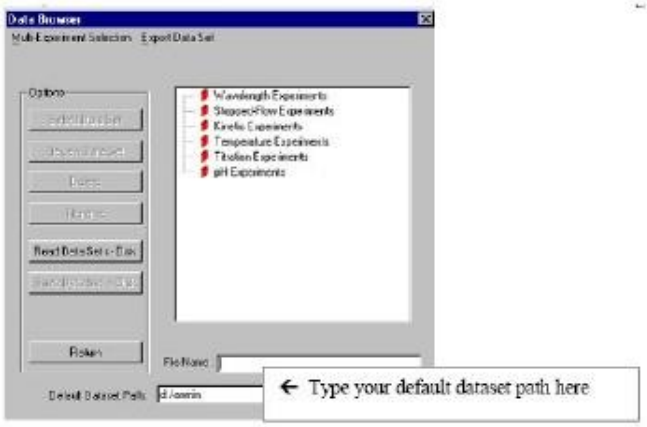
6.4 Sample Preparation

Always prepare chemical solutions away from the computer and spectrometer. Take special care not to spill liquids when they must be brought close to the spectrometer (such as the cuvette).

1. If the sample requires dilution, it should be vortexed for ~20 seconds following dilution to ensure complete mixing, and then centrifuged in an eppendorf tube for ~30 seconds (with a mini-centrifuge) to settle any particulate.
2. All samples containing lipid must be centrifuged in an eppendorf tube for ~30 seconds (with a mini-centrifuge) to ensure that no particles are suspended. Suspended particles will cause unwanted spectral noise due to scattering.
3. Fill a clean cuvette with sample:
    a. Wash the cuvette with pure water (Milli-Q, 2x) followed by methanol (2x), and dry with a stream of nitrogen. You may need to use an eppendorf pipette to remove the water from the cuvette. Be careful when drying with nitrogen, as the pipette attached to the nitrogen cylinder can scratch the cuvette. Do not wipe with a kimwipe as this can also scratch the cuvette.
    b. Use an appropriate size pipette to dispense sample into the cuvette; dispense sample down the side of the cuvette to avoid bubbles, fill the cuvette approximately 90% full, cap.
4. If possible, it is best to run UV scan on a sample prior to doing CD to assess the concentration/intensity of the sample, and avoid too much noise.
    a. The ideal level of absorbance is 0.868 abs, but an absorbance between 0.5 and 1.5 abs is acceptable. High absorbance will lead to increased noise and longer scanning time.
    b. If the UV intensity of the sample is too high, it should be diluted or placed in a cuvette with a shorter path length.

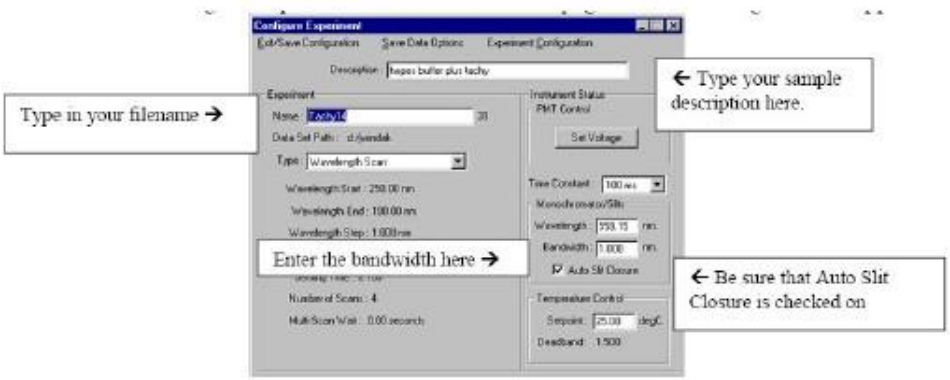
6.5 Recording a CD Spectrum

1. Note: You must press the Enter key after changing any parameter; otherwise the software will ignore the change.
2. Set up a file for your results under the AVIV folder.
    a. In the AVIV software, click on Display and select Data Browser to change where data is stored, and change it to your folder (i.e. \Aviv\YOUR NAME\THE DATE). Press ENTER and THEN press OK.



b. Under “Configure Experiment”:

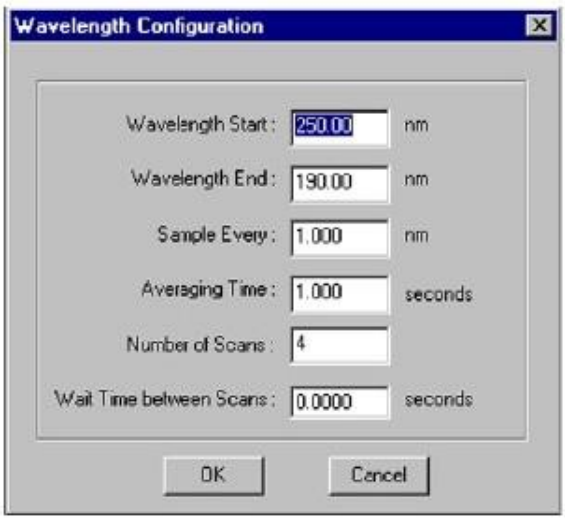
- In the "Description:" box type a descriptive comment for each spectrum you record. This comment should be as detailed as possible.
- Under “Experiment”:
    1. Enter the file name for your set of spectra in the "Name" box.
    2. Select the type of experiment (i.e. Wavelength Scan).
- The "Time Constant" value should normally be 100 ms.
- Under "Monochromator/Slits":
    1. Set the desired wavelength for the experiment.
    2. Enter the desired spectral bandwidth in the "Bandwidth" box (usually 1 or 1.5nm).
    3. Make sure that the “Auto Slit Closure” box is checked.
- Under "Temperature Control” type the temperature for the experiment in the “Setpoint:" box. The temperature can be set at any value between 0 and 90^{o}C.



c. Select "Experiment Configuration" from the top menu:

- Set the "Set Experiment Counter", to 1 (unless you are continuing with a previous set of spectra). The filenames specified in step 2(b) will have #1, #2, etc. appended to them after each spectrum.

- Select "Wavelength Configuration". Fill in the values as appropriate for your experiment, and press OK when finished. [Protein secondary structure is typically assessed in the far UV (190 to 260nm), while the near UV (250-350 nm) can be sensitive to aspects of tertiary structure such as aromatic amino acids and disulfide bonds].



d. Select "Save Data Options" from the top menu:
   - Check the boxes for all the kinds of data you want saved in the file. "CD Signal" and "CD Dynode" should always be saved. Click on OK.
   - The next window which pops up is named "Data Collection Trace Configuration". Check the boxes of which data you want shown on the screen during data collection. This does not affect the data stored in the data file. Press "Enter" when done.

e. Choose the menu choice "Exit/Save Configuration". You will be returned to the main AVIV program menu.

3. Open the lid of the sample compartment. DO NOT rest the CD lid onto the fluorescence source (which is on the far right side of the instrument).
4. Place the filled cuvette into the appropriate adaptor and put the adaptor into the jacket; push it down gently all the way. The cuvette should be oriented in the adaptor the same way for each sample analyzed.
5. Wait for a few minutes to allow the temperature of the sample to equilibrate with the jacket temperature.
6. Start the data collection by clicking on the blue “Run Experiment” on the main page. (If the cuvette holder has been put in backwards the results will be very messy; stop the scan, then turn it around and press start again).

a. The instrument will scan the sample a number of times according to the experiment configuration.

b. The Dynode at the bottom of the screen indicates the signal-to-noise ratio; if it goes above 500V the results are unreliable. If this is the case, stop the scan, change the configuration parameters and re-run the scan.

7. After each spectrum a window appears to determine whether or not you want to save or discard the most recent scan. Choose Store and Save Experiment to Data Browser and Hard Drive if you want to keep the scans.
8. Take out the analyzed sample and put it back in its tube; save the sample in the fridge until you are sure you won't need it.
9. In addition to the samples, a baseline/blank should be recorded, with only the solvent or buffer in the cell. Remember to put the cell in the compartment with the same orientation for each spectrum.
10. When finished recording spectra, follow the instructions for powering down the instrument in section 6.8.
11. To maximize lamp life and nitrogen, calculations should be done with the instrument powered down and using the CD-215 offline software (See section 6.8). Calculations can be performed with the "Math Operations" menu (i.e. subtract solvent baseline, conversion of the raw spectra to standard units such as "Molar Ellipticity", or use the dynode voltages to calculate the absorption spectrum of the sample).

6.6 Setting up the pH meter

If you would like to conduct an experiment using the pH meter, please contact the Instrumentation Technician for specific training on this procedure, as it is not covered in the general training required for the CD Spectrometer.

6.7 Titration Experiments

If you would like to conduct a titration experiment, please contact the Instrumentation Technician for specific training on this procedure, as it is not covered in the general training required for the CD Spectrometer.

6.8 Powering Down the Spectrometer

1. WRITE the lamp hours in the log book.
2. Turn off the lamp power and chiller cooling switch.
3. Quit the software and shutdown Windows.
4. Turn off the CPU and instrument switch.
5. Purge the system with nitrogen for 5 minutes, and then close the valve on the nitrogen cylinder.
6. Clean the cuvettes with methanol followed by pure water and dry with nitrogen before storing.

6.8.1Data Analysis

Once a plot has been drawn, you can use several buttons on the toolbar to manipulate the graph.

1. Switch on the CPU from the main power switch, and then turn on the computer.
2. Navigate to CD-215 offline software.
3. Select the desired data from the sample that have just been run by choosing "Axis Definitions" from the top menu bar, and selecting "Left- Multi-Data Set" OR choose File Load Data Set Read Data.
4. To view the results, Select Displays Data Browser Data Review Wavelength.
5. To average the scans:
   a. From the Left Multi Data Set, choose all the scans (only the scans) and average the data to obtain one graph, using Data Review Average.
   b. Do the same for the baseline files.
   c. Save all data, giving each a separate name under the same file.
6. To remove the baseline from the sample scan:
   a. Select Math Operations (Wavelength Experiments) and perform a subtraction of Data A= sample from Data B=baseline.
   b. Arrange the Left and right axis to be the same, by right clicking on the graph on the left and right sides. You should see your final subtracted/averaged graph.
7. To smooth the final graph:
   a. To get elliptical data, select Math Operations and choose the Smoothed graph to perform this operation. (Convert to Molar Ellipticity; enter total #aa, concentration of sample, path length). The graph's scale should be in the -/+ve thousands and it should have the same shape.
   b. Save the file as ".TXT" file from the left axis data window, at the top left pull out menu (by Exporting file to ASCII Text).
8. To deconvolute the data and obtain estimates of secondary structure content:
   a. Click on the CDNN shortcut and open your ".TXT" file.
   b. You will be prompted what type of deconvolution you want, choose Molar Ellipticity (the 2nd one), and click OK. Then press Deconvolute.
   c. A table should appear listing the wavelength and percentages (not out of 100% necessarily) of alpha helix/parallel/anti-parallel beta strands/ random coils in sections of the wavelengths.
9. To view data and transfer to Origin7, do the following:
   a. Open the Text file in Excel, (use Space to separate the columns).
   b. Copy all the numbers (only nm and Abs) and Paste into a new spreadsheet in Origin.
   c. To make a Graph: Layer Contents, choose spreadsheet data to plot.
10. Also make sure you have the following in the graph: Title, properly labeled axis, legend, colors, bring all curves to the 0 mark,

Note: See www.avivbiomedical.com for links to data analysis software.

7. CALIBRATION

All calibration tests are conducted by the Instrumentation Technician. A record of these tests can be found in Appendix 4.

7.1 Wavelength Calibration

The wavelength calibration and the CD signal should be tested approx. every 300 hours of instrument use (or once every three months) to insure that the instrument is operating properly, and to check for signals of lamp aging.

7.2 CSA Testing

(1S)-(+)-10-Camphorsulfonic Acid (CSA) is used to test the CD signal by assessing the calibration of CD signal intensity and the linearity of the CD signal.

1. Make up a CSA solution of approximately 1.0 mg/mL in ultrapure (Milli-Q) water: Dissolve 50mg in 50mL of Milli-Q water (record exact values).
2. Calibration of the CSA solution:
    a. Turn on the Cary 50 UV-Vis Spec
    b. Run a water baseline of a clean quartz cuvette from 320 to 240 nm, with a 1 nm bandwidth, 1s averaging time, and 0.5 nm step on the Cary 50 UV-Vis Spectrometer.
    c. Run a spectrum of the CSA solution (approximate concentration of 1.0 mg/ml in water) under the same conditions.
    d. Subtract the baseline from the CSA solution and save.
    e. Find the absorbance peak, and record the wavelength and the absorbance. The expected peak is at 285 nm.
    f. Calculate the expected elliptical signal in millidegrees using the Beer Lambert law: mdeg = 2254.4 x Abs.
3. CD amplitude calibration:
    a. Start up the CD spectrometer as described in section 6.5, and configure the experiment in Kinetics mode; 290nm, 1.0 BW, 1s interval, 1s AVT, and 60 second duration.
    b. Measure the water baseline on the CD. Record the ellipicity at 290 nm
    c. Measure the CSA and record the ellipicity at 290 nm.
    d. Subtract the baseline and from the sample and compare to the value calculated in step 2(f).
    e. If required, adjust the DC gain to value calculated.
4. CSA peak ratio test:
    a. Start up the CD spectrometer as described in section 6.5, and configure the experiment in Wavelength Scan mode; 320 to 180 nm, 0.5 nm step, 1.0 BW, 1s interval, and 1s AVT.
    b. Collect a spectrum of CSA (1 cm pathlength, 1 mg/ml) and rename CSA.
    c. Collect a water baseline, and subtract from the CSA measurement.
    d. Record the actual wavelength and CD of the peaks at 192.5 nm and 290 nm.
    e. Calculate the ratio of CD at 192.5 to CD of 290. This value should be between 1.9 and 2.2.

Table 7-1: The CD properties of d-10-Camphorsulfonic acid in water (1mg/mL, 1mm cuvette) (The Leibniz Institute for Age Research).

	Value ($M^{-1}cm^{-1}$)	Value (deg cm^2 $dmole^{-1}$)
ε_{285nm}	34.5	-
$[\theta]_{290.5nm}$	-	7.800
$[\theta]_{192.5nm}$	-	-15.600
Ratio $(192.5/290.5)_{nm}$	-	2.000

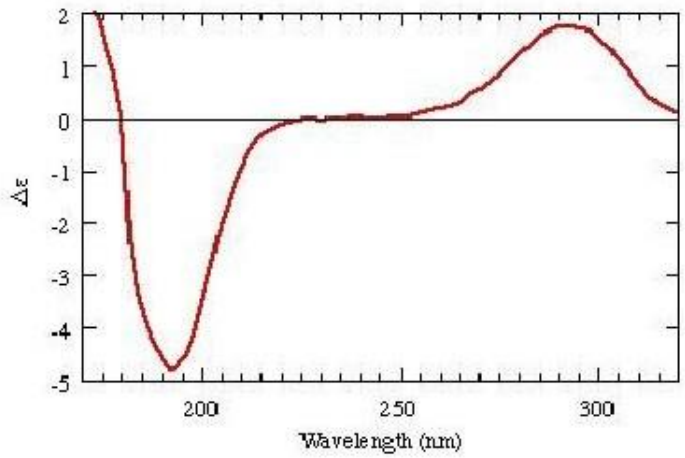


Figure 7-1: The molar CD spectrum of d-10-Camphorsulfonic acid in water (1mg/ml, 1mm cuvette) (The Leibniz Institute for Age Research).

7.3 Energy Test

With the polarizer, modulator, and N purge on high: Record the dynode voltages at 1.0 nm BW for the following wavelengths: 800,700, 600, 500, 400, 300, 250, 225, 200, 190, 187.5, 185, 180, 179, 178, 177, 176, and 175.

7.4 Noise Test

1. Set the polarizer, modulator, and N purge on high. Set at 1.0 nm BW, 500 nm, 0ABS, 1s AvT, 1s step, for 10 minutes.
2. Go to the wavelengths listed below, record a scan, save, record peak to peak noise, and RMS: 500, 200, 285, 280, 278, and 275.
3. The values should be similar to:
   a. 0.06 mdeg at 500 nm
   b. 0.08 mdeg at 200 nm
   c. 0.1 mdeg at 185 nm

8. TROUBLESHOOTING

Table 8-1: Troubleshooting Suggestions

Problem	Cause	Solution
The lamp turned off on its own.	The lamp has overheated, as indicated by the red LED marked TEMP.	Check to make sure the Chiller is turned on and operating properly. If it is, contact the Instrumentation Technician and shutdown the instrument according to section Error! Reference source not found..
The spectrum is very noisy.	The sample matrix may contain compounds or ions which interfere with the spectrum (i.e. Chloride).	Try using a different buffer or matrix if possible (i.e. replaced NaCl with NaFl).
The signal is very weak.	The sample is too dilute.	Try running a more concentrated sample.

9. PREVENTATIVE MAINTENANCE

Users are not to perform maintenance. These procedures are carried out by the Instrumentation

9.1 Daily

- Check the level of liquid N in the cylinder
- Check the log book

9.2 Monthly

- Replace the fluid in the Merlin Chiller if algae growth is apparent
  - Fill with DI water, and add 1 g of chloramine-T per 3.5 L of water (the total volume of the bath is 1.8 L, so approximately 0.51 g of chloramine-T is required)
- Check the fluid levels in the pH probe
-

9.3 Three Months

- Run calibration procedures

9.4 As Required

- The Xe lamp has a lifetime of approximately 1000-1500 hours. The lamp must be replaced when it reaches 1500 hours, or when the stability changes sharply.

10.QUICK REFERENCE GUIDE

1. Ensure you are using clean quartz cuvettes.
2. Check the level of nitrogen in the cylinder and make sure that the cylinder is not close to empty. NOTE: you must remain in the vicinity of the instrument throughout the experiment as the nitrogen gauges can be unreliable.
3. Turn on the nitrogen gas and turn the pressure regulator to the red line (35 psi).
4. Wait approximately 20 minutes while the instrument is purged with nitrogen gas. Check the gas flow meters and the Merlin Chiller while the instrument is purging.
5. Turn on the cooling switch on the chiller (at the front of the chiller).
6. Turn on the lamp power using the power switch below the spectrometer.
7. Wait approximately 30 seconds for the yellow lamp ready light to go on.
8. Fire the lamp by pressing and releasing the RED Start button (below the lamp ready light). Make sure that the lamp is ignited and drawing a current.
9. Record the lamp hours in the log book (hours found on the LED display located below the main instrument).
10. Turn on the instrument switch and the CPU.
11. Start the software using the CD-215 icon on the desktop (no username or password).
12. Let the instrument lamp warm up and equilibrate for 30 to 40 minutes before proceeding with measurements.
    a. During this time the sample can be prepared as follows:
        - All samples containing lipid must be centrifuged for ~15sec (with the mini-centrifuge)
        - Wash the cuvette with methanol followed by pure water, dry with nitrogen.
        - Dispense sample into the cuvette; fill the cuvette approximately 90% full, and cap
        - Run UV scan on a sample prior to doing CD (abs between 0.5 and 1.5 is acceptable).
13. Configure your experimental parameters.
    a. Select Data Browser to change where data is stored, and change it to your folder.
    b. Under “Configure Experiment”: Fill in the "Description" box
    c. Under “Experiment”:
        - Enter the file name for your set of spectra in the "Name" box.
        - Select the type of experiment (i.e. Wavelength Scan).
    d. The "Time Constant" value should normally be 100 ms.
    e. Under "Monochromator/Slits":
        - Set the desired wavelength for the experiment.
        - Enter the desired spectral bandwidth in the "Bandwidth" box (usually 1 or 1.5nm).

- Make sure that the “Auto Slit Closure” box is checked.

f. Under "Temperature Control” type the desired temperature in the “Setpoint:" box.

g. Select "Experiment Configuration" from the top menu:
   - Set the "Set Experiment Counter", to 1.
   - Select "Wavelength Configuration”. Fill in the values as appropriate.

h. Select "Save Data Options" from the top menu and check the boxes for all the kinds of data you want saved in the file. Click on OK.

i. Choose the menu choice "Exit/Save Configuration". You will be returned to the main AVIV program menu.

14. Open the lid of the sample compartment.
15. Place the filled cuvette into the appropriate adaptor and put the adaptor into the jacket.
16. Wait for a few minutes to allow the temperature of the sample to equilibrate.
17. Start the data collection by clicking on the blue “Run Experiment” on the main page.
    a. If the Dynode at the bottom of the screen goes above 500V the results are unreliable.
18. Store and Save Experiment to Data Browser and Hard Drive if you want to keep the scans.
19. Take out the analyzed sample and put it back in its tube, save the sample in the fridge until you are sure you won’t need it.
20. In addition to the samples, a baseline should be recorded, with only the solvent in the cell.
21. WRITE the lamp hours in the log book
22. Turn off the lamp power and chiller cooling switch.
23. Quit the software and shutdown Windows.
24. Turn off the CPU and instrument switch.
25. Purge the system with nitrogen for 5 minutes, and then close the valve on the nitrogen cylinder.
26. Clean the cuvettes with methanol followed by pure water and dry with nitrogen before storing.
27. To analyze data, switch on the CPU from the main power switch, and then turn on the computer. Navigate to CD-215 offline software.

11.REFERENCES

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APPENDIX 1: USER LOG

DATE	NAME	EXT #	SUPERVISOR	LAMP HOURS AT START	CD-PMT READING AT 200 NM	LAMP HOURS AT END	PROBLEMS/CONCERNS

APPENDIX 2: CALIBRATION LOG

DATE	NAME	EXT #	TEST TYPE	RESULTS	PROBLEMS