

Chirascan Quick Start

This guide provides an introduction to the use of the Chirascan CD Spectrometer.

It covers the running of simple experiments and the basic use of the software.

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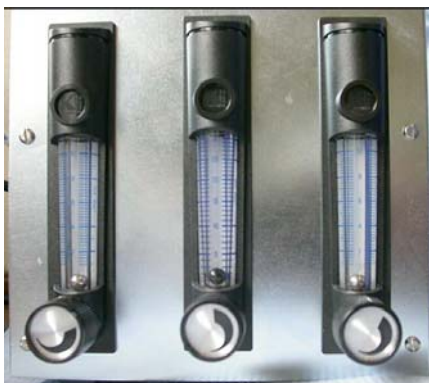
2.0 Introduction

APL recommends that new users read this guide to familiarise themselves with the basic operation of the instrument. The operations covered are start up of hardware and software, use of the Chirascan instrument control and data acquisition software to collect CD spectra and baselines, use of the graphics software to carry out common operations on the data and to save data appropriately and conversion of data formats for export to third-party programs.

2.1 Purging

Removal of air from the light path by purging with nitrogen is essential, not optional. Failure to purge the instrument will result in the formation of ozone, which is damaging to health and can cause deterioration of the optical components in the instrument.

The instrument is purged using clean nitrogen that is distributed to the instrument via a manifold that contains three gas flow controllers (see picture below). The gas pressure regulator of the master supply should be set in the range $2\text{bar} < P < 6\text{bar}$, in accordance with the manufacturer's recommendation. At first start-up, set the gas flow as high as the supply pressure allows, with most flow through the monochromator (middle) flow controller, and let the purge to continue for at least 15 minutes prior to attempting to ignite the lamp (see next section). After this initial, high-flow purge, the controllers can be set at 1 Lmin^{-1} , 3 Lmin^{-1} and 1 Lmin^{-1} for the lamp housing, monochromator and sample housing respectively.



Three rotameters (gas-flow controllers) control nitrogen flow to the lamp housing, monochromator and sample housing respectively, from left to right.

The lamp housing and monochromator are hermetically sealed, which means that once the initial purge is complete, you should not need to repeat the high flow-rate purge, unless the hermetic seals are broken (during service or lamp replacement) or if the instrument has been left idle for many days.

NB It is essential that the nitrogen used to purge the instrument be clean. APL strongly recommends the use of a combined molecular sieve (X13) and activated charcoal gas trap, which will remove from the nitrogen stream all common impurities, which can otherwise deposit on the internal optical surfaces. The cleaner the nitrogen, the longer your Chirascan will maintain its far-UV performance.

2.2 Power-up

Ensure that the instrument is connected to the electricity supply and that it has been purged according to Paragraph 2.1. Put the two black rocker switches (System and Lamp) on the front panels to the on (I) position. The instrument will automatically check its zero positions (you may hear the monochromator motors moving). Ignite the Xe arc lamp by pressing the red Start button on the lamp control panel *no sooner than 20 seconds* after the lamp power supply has been switched on – this is to allow sufficient time for the capacitors to charge up. If ignition is successful, the translucent panel on the source housing adjacent to the control panel will glow yellow; if you do not see a yellow glow, press the red Start button again; repeat until ignition is achieved. The lamp will require at least 15 minutes to stabilise following ignition. Finally, ensure that the computer, its monitor and printer are all powered up.



The System panel

NB Once the lamp is struck, it is strongly recommended that it be left on for the duration of the work planned for the day. Repeated powering up and powering down will lead to instabilities (see previous paragraph) and will reduce the life of the lamp. However, if it is necessary to restart the lamp after running, it must be allowed to cool for about 15 minutes before re-ignition is attempted.

The light-emitting diodes (LED) on the System panel are labelled Status, Tx and Rx; Tx and Rx will illuminate when commands are being transmitted or data is being received respectively; the green Status LED should be constantly illuminated – if it is flashing, it indicates that one or more of the electronics self-tests has failed. If this happens, try restarting the electronics and if the problem persists, please contact APL technical support.



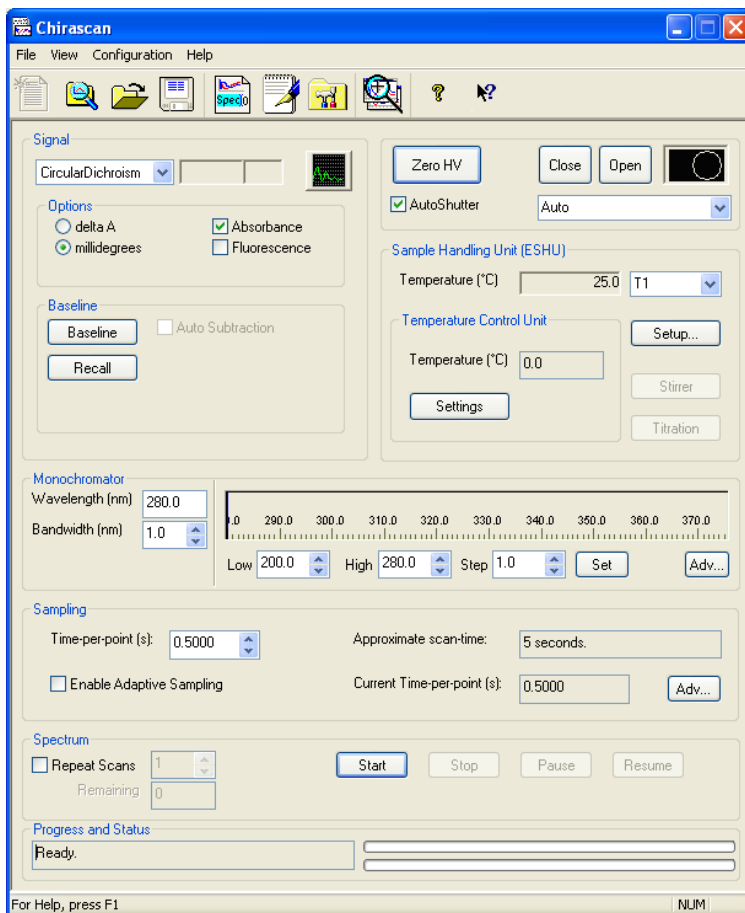
The Lamp panel

The red LED on the Lamp panel indicates that power is present. The digital timer records the number of hours that the lamp has been running; adjacent to the timer is a recessed reset button: the timer must be reset when the lamp is changed.

On shut-down, it is recommended that only the lamp be extinguished by putting the black rocker switch to the off (O) position. The electronics can be left switched on quite safely. Once the lamp is extinguished, the nitrogen should be left on for a few minutes while the lamp cools and because the lamp housing and monochromator are hermetically sealed, there will be little or no loss of purging integrity overnight or even over a weekend. Resuming normal nitrogen flow for 10 minutes after a weekend shutdown is usually sufficient to re-establish a fully purged light path.

2.3 Launching the software

On starting the computer, among other icons on the desktop, you will see two associated with the Chirascan. They are called Chirascan and Chirascan Viewer. Double-click on the Chirascan icon to launch the Pro-Data interface.



The Pro-Data interface

The Pro-Data interface enables you to configure the instrument and to manage experiments. It is divided into a number of sub-panels - *Signal*, *Baseline*, *Sample Handling Unit*, *Monochromator*, *Sampling*, *Spectrum* and *Progress* - in which are grouped the parameters relevant to the control or management of that sub-panel's

function. In addition, there is an unlabelled panel for controlling shutter function and detector high voltage.

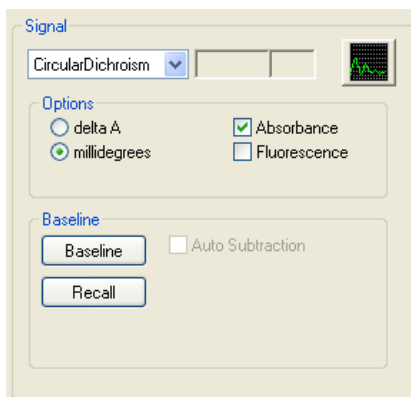
2.3.1 Measuring a CD baseline and spectrum

NB When you put a sample into the sample chamber, it is inevitable that a small quantity of air will be introduced to the low-volume light path within the purge tubes in the sample chamber. It takes only a few seconds to re-establish an air-free environment and you should not need to wait longer than that before starting your measurement.

2.3.1.1 The CD baseline

Every CD spectrometer has a CD baseline, caused by the interaction of circularly polarized light (CPL) with the slight birefringence found in the PMT detector window and to a lesser extent in the cuvette. To see the true CD spectrum of your sample, this baseline must be measured and subtracted from the CD spectrum of the sample.

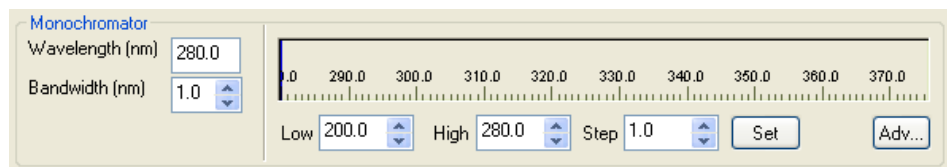
To measure the CD baseline, you must measure the CD spectrum of a blank. The blank must be identical in every way to the sample that you want to measure, except that the sample itself is absent. For example, if you intend to measure an aqueous solution of lysozyme in a 0.2mm path-length rectangular cuvette, then the CD baseline is determined using the same cuvette containing only water.



Signal panel in the Pro-Data interface

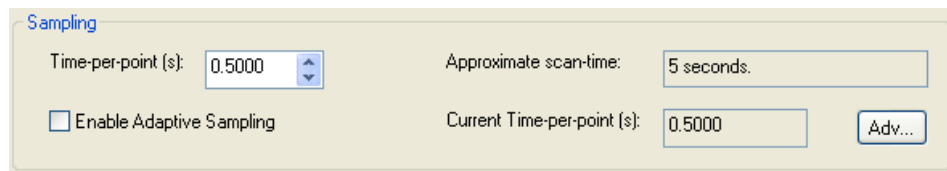
In the *Signal* panel, there is a drop-down menu that contains the main data collection modes: choose *Circular Dichroism*. In *Options*, choose either *delta A* (delta absorbance) or *millidegrees*, depending on the units in which you wish to express the CD measurement. Select *Absorbance* if you wish to simultaneously measure the absorbance

spectrum of the sample. Note that *Circular Dichroism*, *millidegrees* and *Absorbance* are selected by default.



Monochromator panel in the Pro-Data interface


Set the lower and upper wavelength limits of the scan range for your experiment in the *Monochromator* panel of the Pro-Data interface – these will normally be the same for both CD baseline and CD spectrum. For example, set *Low* to 180nm and *High* to 260nm if you are measuring a typical protein. Set the wavelength step size in *Step*, typically 1nm or less, and click on *Set*. To the left of the monochromator wavelength bar, set the required bandwidth. Typically, a bandwidth of 1nm is suitable for protein CD spectroscopy.

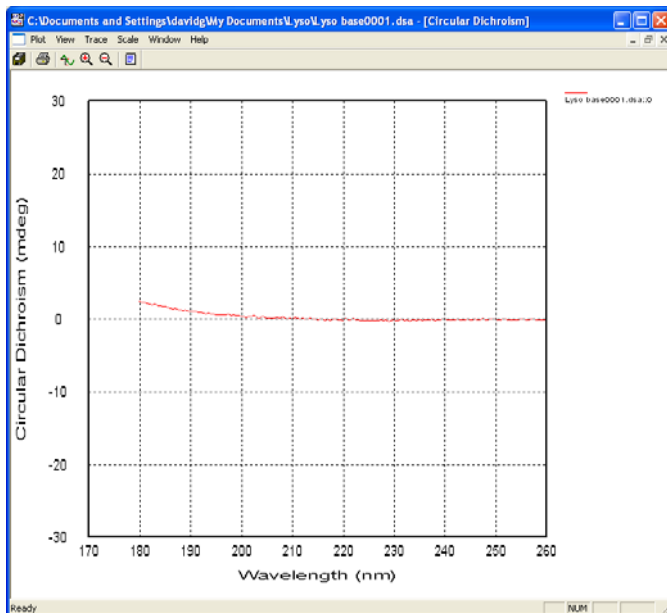


Sampling panel in the Pro-Data interface


In the *Sampling* panel, set the time per point to the desired value. An approximate scan-time is shown, which is approximately equal to the product of the time-per-point and the scan range divided by the step size. Note that the scan time will have a direct effect on the quality of the spectrum. If you are happy with this, with the blank in place in the sample holder, go to the *Signal* panel and click on *Baseline*. A CD baseline will now be measured; the display and data manipulation program Pro-Data viewer will automatically start and the data will be displayed; the data are stored as a file called Baselinexxxx.dsa (unless a different seed name has been specified – see later) in your default working directory. The *Auto Subtraction* tick box becomes active at the conclusion of a baseline scan: if you wish to subtract the baseline automatically from subsequently measured CD spectra, tick *Auto Subtraction*.

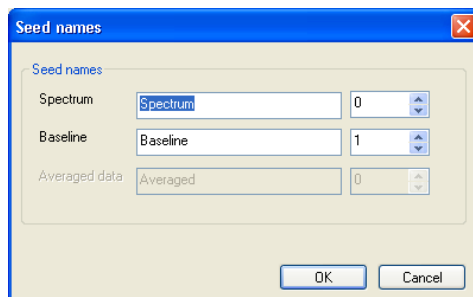
NB. If Pro-Data viewer has been launched from the desktop independently of Pro-Data itself by clicking on its desktop icon, then Pro-Data will not know of the existence of the viewer – it is a separate program – and Pro-Data viewer *will not respond to the start of an experiment in the normal way*.

In general, when working at the instrument, it is recommended that you launch Pro-Data Viewer from Pro-Data using the  icon in the toolbar. This will ensure that local communication is established, that Pro-Data viewer will work correctly as a real-time display and that the data will be saved to a DataStore in the current working directory.



Baseline CD spectrum

You do not need to use the default name for the baseline – you can specify a seed name by clicking on the *Seed Names* icon  in the toolbar of the Pro-Data interface and specifying seed names for the baselines and the spectra.

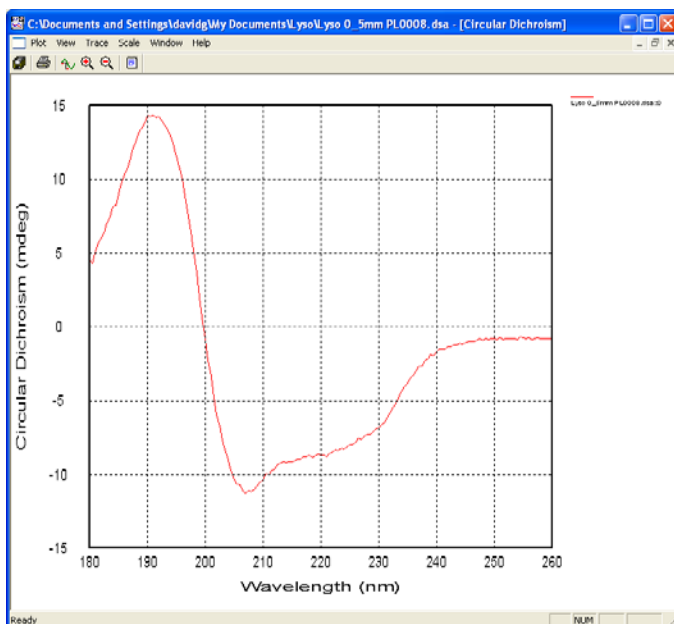


Type in seed names of choice

2.3.1.2 The CD spectrum

Once you have measured the CD baseline, replace the blank with your sample. As the conditions for the measurement of the sample will be identical to those for the blank, go straight to the *Spectrum* panel of the SPC and click on *Start*. The CD spectrum will be recorded under the conditions applied to the blank and stored as a file called Spectrumxxxx.dsa, unless you have specified a different seed name (see previous paragraph).

The program Pro-Data viewer will automatically display the data as it is collected; an example is given below. Double-clicking anywhere in the graphics panel will autoscale the display.

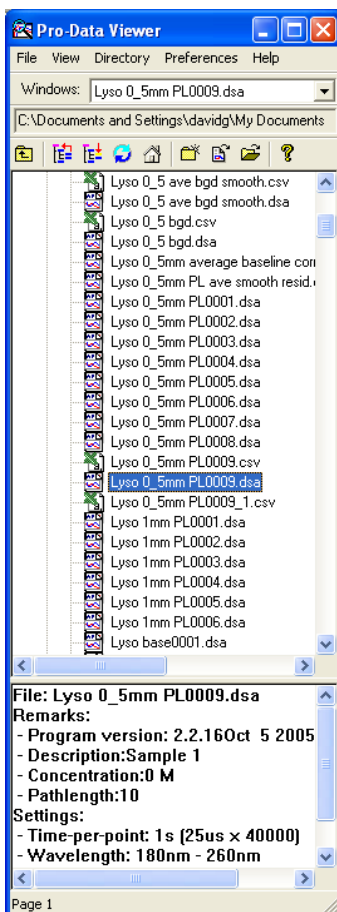


CD spectrum of lysozyme (raw data)

When measuring any CD spectrum on Chirascan, other properties are simultaneously recorded. These include the voltage applied to the detector, the temperature and the absorbance spectrum of the sample. They can be viewed by selection from the *Window* menu.

2.4 Viewing and manipulating spectra and traces

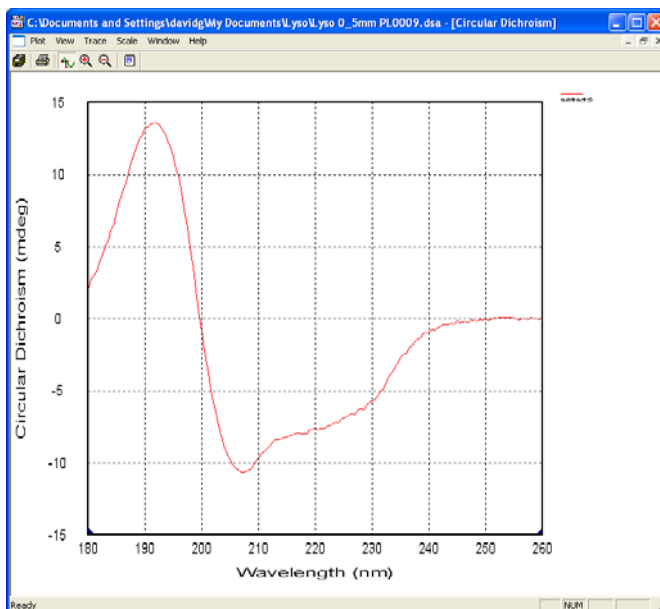
If you have just measured a CD spectrum, the Pro-Data viewer launchpad and graphical display will be running and the CD spectrum will be displayed. If Pro-Data viewer is not running, launch it using the icon in the Pro-Data interface toolbar or, if the Pro-Data interface is not running either, from the desktop icon (see note at end of section 2.3.1.1). The Pro-Data viewer launchpad will appear on the screen, open at the current working directory. You can change the location of the working directory by selecting or creating a different folder and using the *Set Working Directory* option from the *Directory* menu. The working directory is where new spectra will be saved.



The Pro-Data viewer launchpad

2.4.1 Viewing spectra

With the Pro-Data viewer launchpad open, navigate in the usual way until you find the file of interest and double click on it. In the above figure, the file 'Lyso 0_5mm PL0009.dsa' has been chosen; Pro-Data viewer graphical display opens and displays the data contained in the file (below).



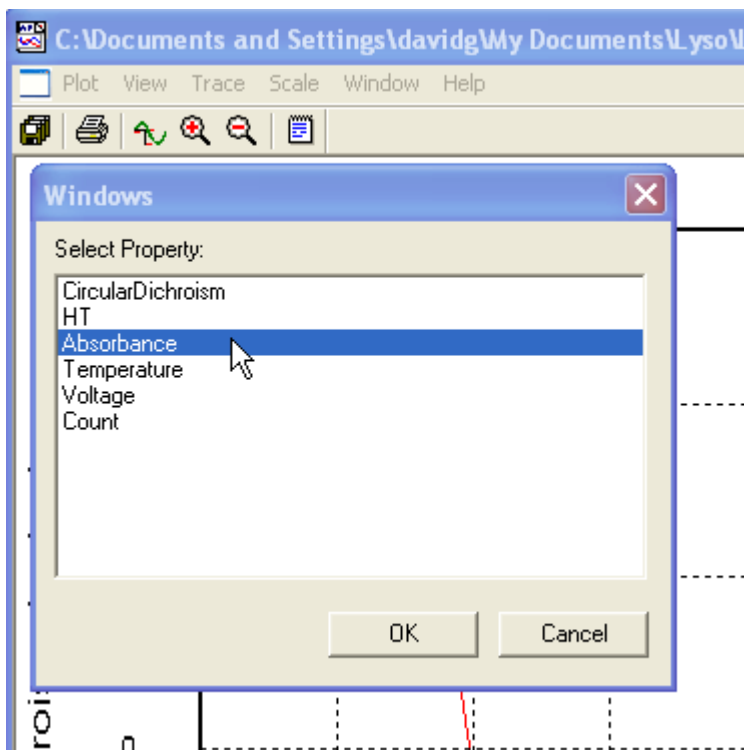
CD spectrum of lysozyme (baseline corrected, raw data).

Double-clicking the left mouse button anywhere on the graphical display will autoscale the axes such that you see the full spectrum. To zoom in to a particular part of the display, click and hold the left mouse button whilst moving the cursor to create a box around the area of interest. On releasing the left mouse button, the newly-created box will expand to fill the display window. To return to the original display, simply double-click the left mouse button again.

To zoom out, press the shift key and simultaneously click and hold the left mouse button whilst moving the cursor to create a box. On releasing the left mouse button, the entire spectrum will be rescaled to fit within the newly-created box. To return to full display, simply double-click the left mouse button again.

Other properties are measured simultaneously with the CD spectrum (see Section 2.3.1.2 above). To examine these results in Pro-Data viewer, select the property of

interest, e.g. absorbance, from the *Window, New Window...* menu. Alternatively, right-click on the y-axis label and choose the property from the pop-up menu that appears.




Choosing another property – here, absorbance will be displayed

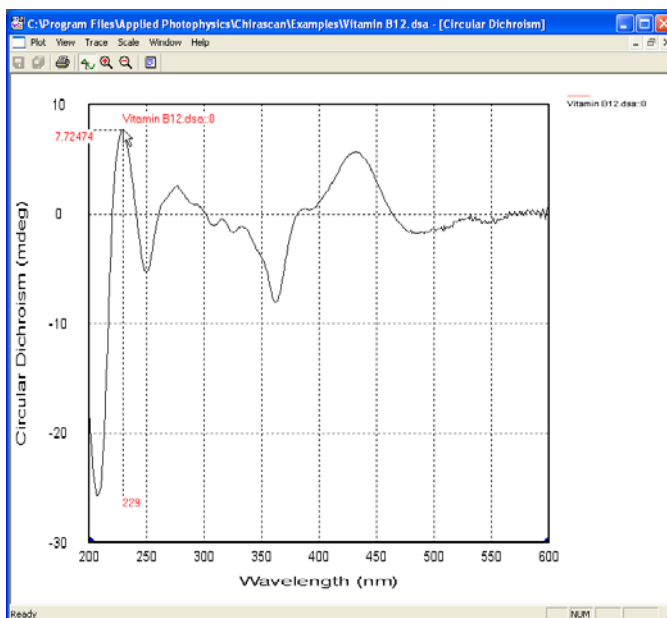
2.4.2 Comparing spectra

If you have collected some spectra that you wish to compare, simply drag-and-drop the files of interest to the graphical display window. You can do this from the launchpad of Pro-Data viewer. The spectra will be displayed in a single window. Note that the data sets must be compatible – you cannot overlay a trace collected as a function of wavelength with one that was collected as a function of some other property, such as temperature nor, currently, if the wavelength ranges and step-sizes are not identical.

2.4.3 Manipulating spectra

There are simple rules governing the manipulation of spectra in Pro-Data Viewer. Firstly, choose *Selection On* from the *Trace* menu. Alternatively, you can click the

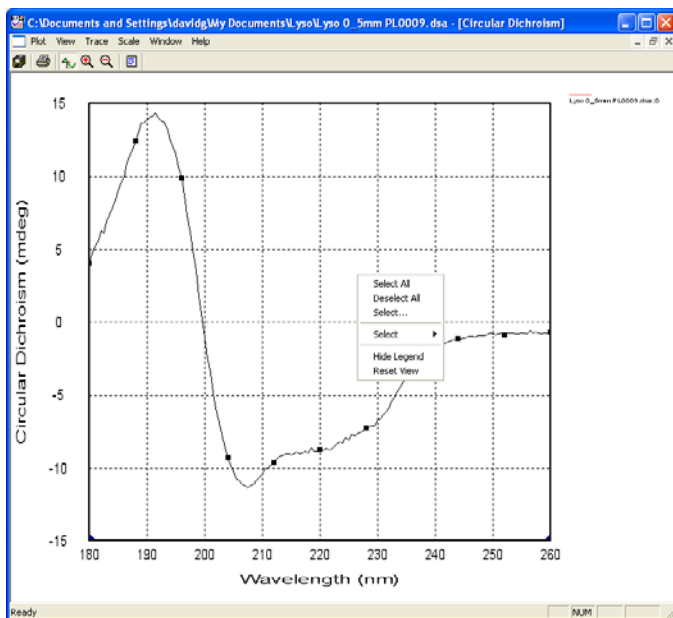
Trace Selection icon  from the toolbar or toggle selection on and off using the space-bar on the keyboard when the display window is active. Any of these methods can be used to activate trace selection. Then move the mouse cursor to the graphical display. If *Show Coordinates* and *Show Trace Name* are selected from the *Trace* menu (default and recommended), you will note that by positioning the cursor over a spectrum an identification string appears next to the cursor together with the coordinates of the corresponding data value. The trace itself is highlighted by a change of colour.



Trace selection

To select a highlighted trace for manipulation, click the left mouse button. ‘Selection marks’ will appear along it; move the cursor away from the trace and click the left mouse button again to deselect it. To make multiple selections, repeat this process whilst depressing the *Ctrl* key. (Note to select all traces, a shortcut is available – with the cursor in the graphics panel, right-click the mouse and choose *Select All* from the pop-up menu.) To deselect a trace when more than one has been selected, highlight the trace to be deselected and click the left mouse button while holding down the *Ctrl* key.

With one or more traces thus selected, click the right mouse button anywhere in the display panel to open the select mode.

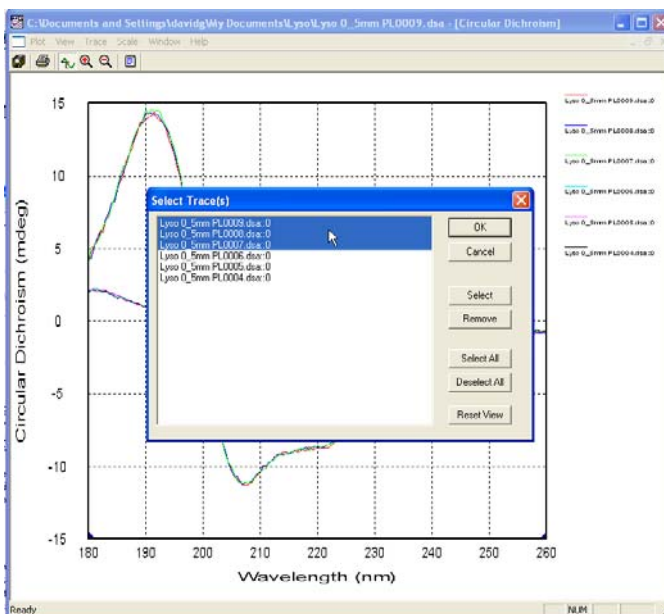


Trace selected (note marks), right-click mouse button to reveal pop-up menu.

Click on *Select All* if you wish to select every trace that is displayed; click on *Save All* if you wish to save every trace that is displayed, regardless of selection; click on *Reset View* if you wish to restore only the original data to the display.

Once a trace has been selected, if there is more than one trace displayed, you can use the up/down (μO) arrows on the keyboard to move between traces.

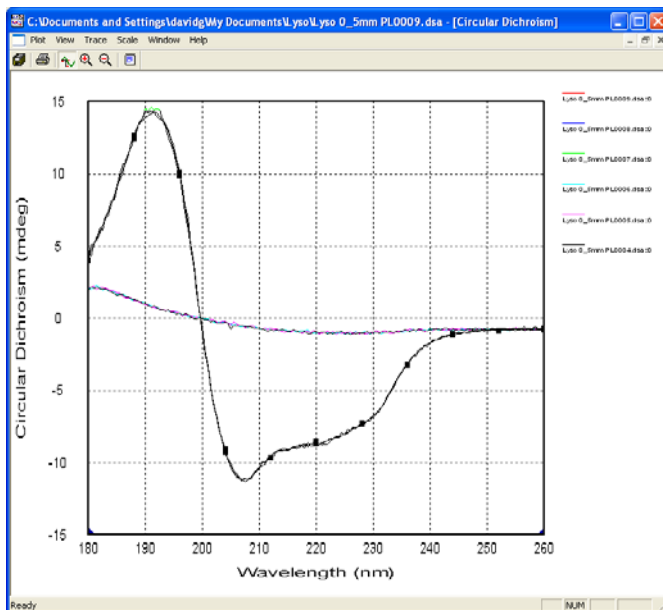
The method of selection described thus far is perfectly good for dealing with a limited number of traces but can become a little cumbersome if there are many traces simultaneously displayed especially if they overlay, for example if you have measured ten repeats of the same sample and wish to correct for baseline and then average and smooth (see below for a description of these operations). Under such circumstances, it is recommended that the *Select...* option is chosen from the drop-down menu and that traces be selected from the list thus presented.



Selecting traces using the Select... drop-down menu.

In the above figure there are six traces displayed, three overlapping spectra and three overlapping baselines. To select a trace, simply click on the trace name and then on *OK* or *Select*. To select more than one trace, hold down the *Ctrl* key and click on the trace names of interest or, if the traces appear in the list sequentially, select the first one and then hold down the *Shift* (*V*) key and select the final one in the list. To select all traces in the window, choose *Select All*. To deselect an individual trace from a selection, hold down the *Ctrl* key and click on the trace name; to clear your selection completely, click on *Deselect All*; to remove traces from the display, click on the trace names and then on *Remove*; to reset the display to its original state, click on *Reset View* but note that the original state will be the first file that was opened. If a trace was selected prior to opening the *Select...* menu, then it will be highlighted in the list already.

Once you are happy with the selection, click on *OK*. In the above figure, three trace names have been selected and the result is shown below.



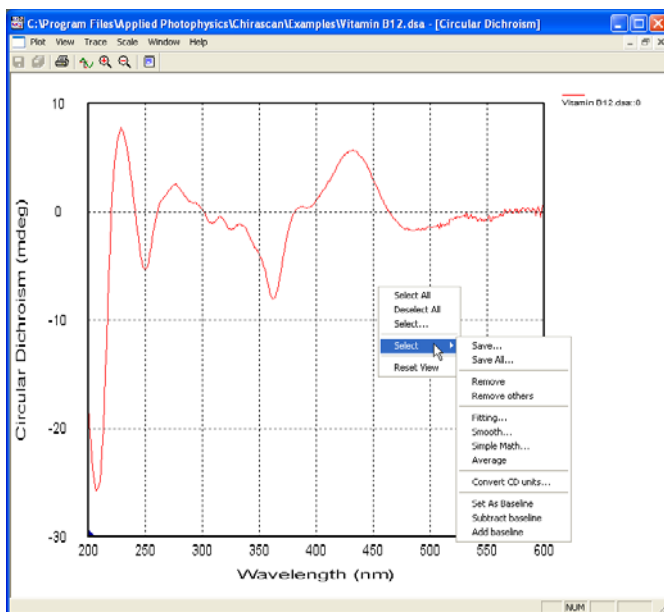
Three spectra selected

Once the traces are selected, choosing the *Select* sub-menu (below) will enable you to carry out certain operations on the selected traces.

Click on *Save* if you wish to save the selected trace or traces, which will be saved to a new file. You will be asked to choose the name and location of the file that contains the results as part of the process. Note that the original data is always left intact. Click on *Save All...* if you wish to save every trace in the display, regardless of whether or not it has been selected.

Click on *Remove* if you wish to remove the selected trace or traces from the display; click on *Remove others* if you wish to remove from the display all traces that are *not* selected.

Click on *Set As Baseline* if you wish to define the selected trace as a baseline. The solid line of the selected trace will change to become a dotted line, so that it is easily identified.



Example displaying the Select sub-menu

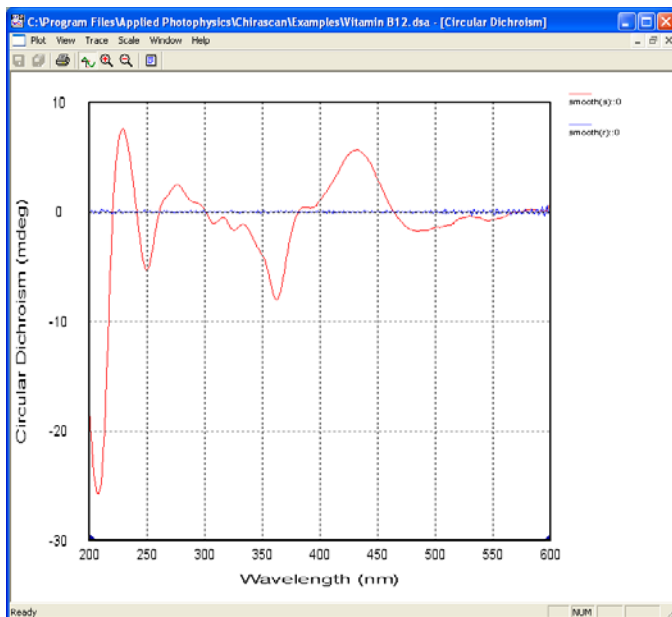
Click on *Subtract baseline (Add baseline)* if you wish to subtract (add) a previously defined baseline from the selected trace or traces. A new trace or set of traces will appear in the display window: these are the baseline-subtracted (added) spectra. **NB** the only reason that you might wish to add a baseline is if you have previously subtracted one in error.

Click on *Average* if you wish to create a new trace that is the average of the selected traces.

Choosing *Smooth...* in the *Select* sub-menu opens a dialogue box that enables you to execute a Savitsky-Golay polynomial smooth on the selected trace or traces. The original trace, the smoothed trace and, importantly, the residual trace (original minus smoothed) will appear together in the display window. **The residual trace or traces must be inspected.** If the residual noise is randomly distributed about zero, then the smooth has not distorted the data; if there is any non-random structure, then the smoothing process has introduced some distortion of the spectral features and the result should be discarded.

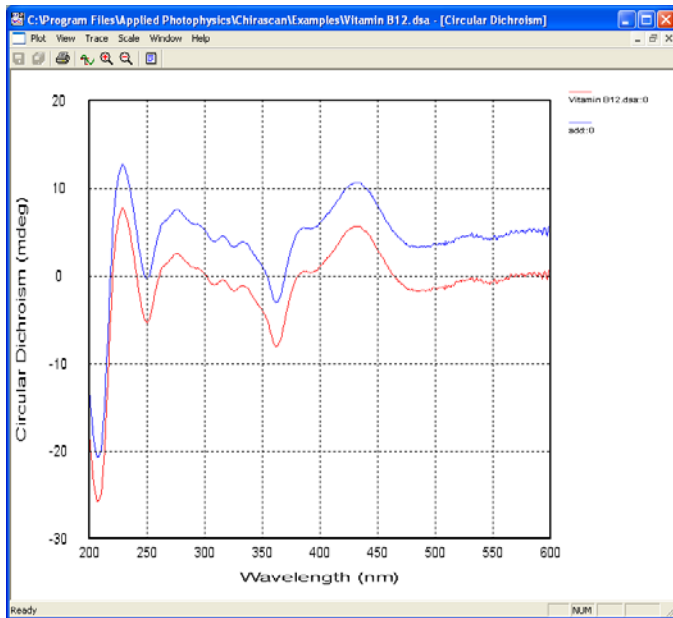
The larger the number of points used in the Savitsky-Golay smooth, the greater will be degree of smoothing. The optimum value will depend on the measurement resolution

and fine structure of the spectrum. It is important that inspection of the residual be carried out to ensure that an appropriate smooth has been applied. As the original raw data is left intact, the cycle can be repeated and the number of points quickly optimised.



Smoothed spectrum of vitamin B12, showing residual randomly distributed about zero.

Choosing *Simple Math...* in the *Select* sub-menu opens a dialogue box that enables you to perform simple arithmetic operations on the selected trace or traces. Typically, this is used to offset a selected trace by adding a constant. The constant is in the units of the y-axis. Another example is to apply a scaling factor to a selected trace.



Spectrum of vitamin B12, original (red) and offset by 5mdeg (blue).

Choosing *Convert CD Units...* in the *Select* sub-menu opens a dialogue box enabling you to convert the units in which the circular dichroism is expressed. For example, if you made your measurement in millidegrees, open the drop-down menu to choose from delta absorbance (ΔA), mean residue molar ΔA , mean residue molar ellipticity, molar ΔA or molar ellipticity. The edit boxes *Concentration (M)*, *Path Length (cm)* and *Number of Residues* are active or greyed out according to whether or not that particular input is required. You will be asked to save the converted data as a new file.

The image shows a dialog box titled "Convert CD Units" with a close button (X) in the top right corner. The dialog contains the following fields and controls:


- Convert From:** A text box containing "mdeg".
- Convert To:** A dropdown menu with "mean residue molar delta A" selected.
- Concentration (M):** A text box containing "0".
- Path Length (cm):** A text box containing "0".
- Number of Residues:** A text box containing "1".

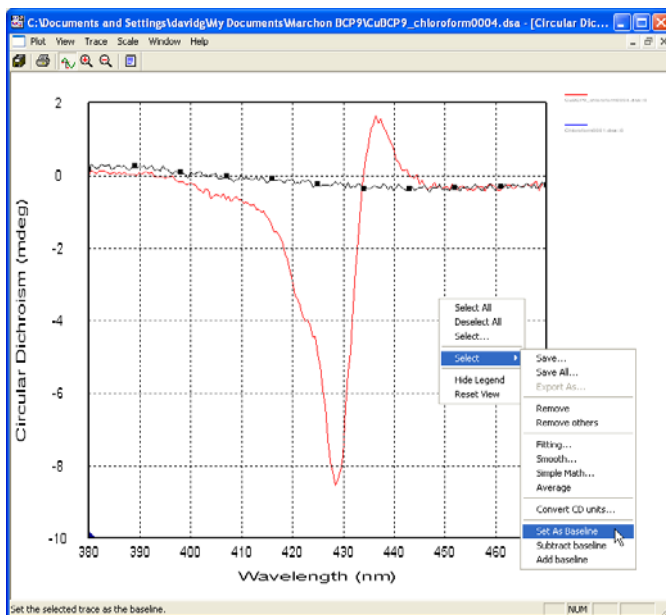
At the bottom of the dialog are two buttons: "Cancel" and "OK".

2.4.4 Example of spectrum manipulation

A typical experiment will yield a CD spectrum and the corresponding CD baseline. Data manipulation might well involve baseline subtraction followed by a Savitsky-Golay smooth. The operations are described below.

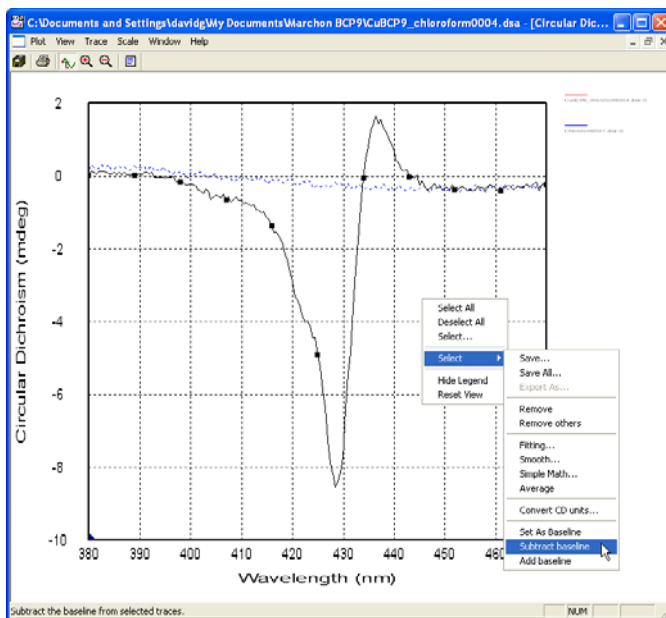
With Pro-Data viewer open, double click on the file that contains the CD spectrum data – the file will open and the data will be displayed. Then drag and drop the corresponding CD baseline data onto the display window. A warning will appear if the files are incompatible. Assuming that no previous baseline subtraction has occurred,

switch on Trace Selection  (or hit the space-bar), select the baseline trace and right-click the mouse anywhere in the display panel. Select *Set As Baseline* from the *Select* sub-menu of the pop-up menu. The trace will change appearance and become a dotted line.



Setting a trace as baseline.

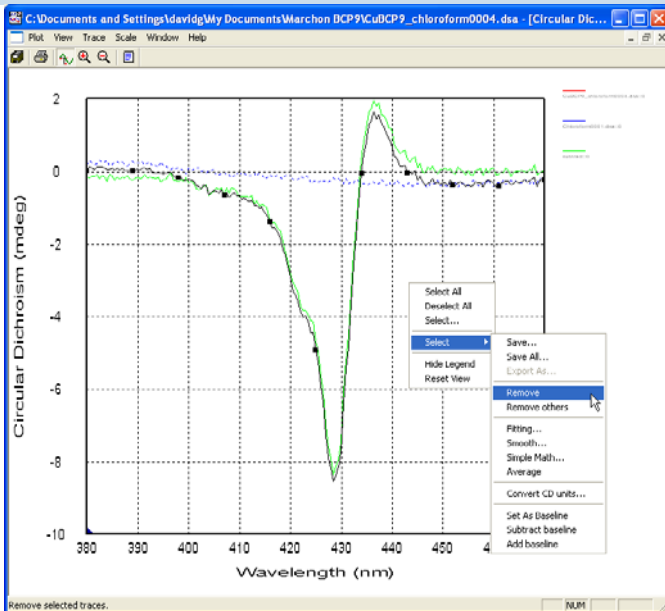
Now select the trace from which you wish to subtract the baseline and choose *Subtract baseline*. A third trace will appear - the baseline-subtracted CD spectrum.



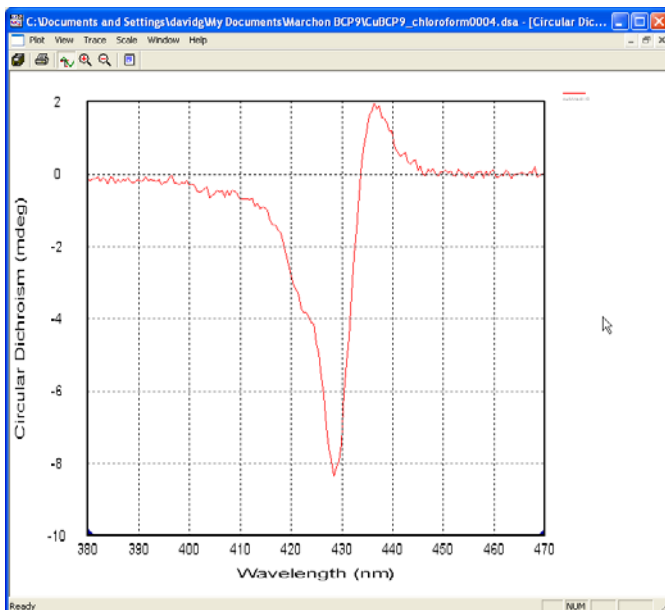
Subtracting a baseline from a spectrum.

To leave the baseline-subtracted spectrum only on the display, select the original spectrum and using the drop-down menu, click on *Remove*. The original spectrum and the baseline will disappear, leaving the baseline-subtracted spectrum only.

You can save any data at any time – if you wanted to save all the data that are represented in the above figure, simply select *Save All* from the pop-up menu and follow the usual procedure. If you only wish to save a selection, choose the *Select* sub-menu and *Save* option instead.

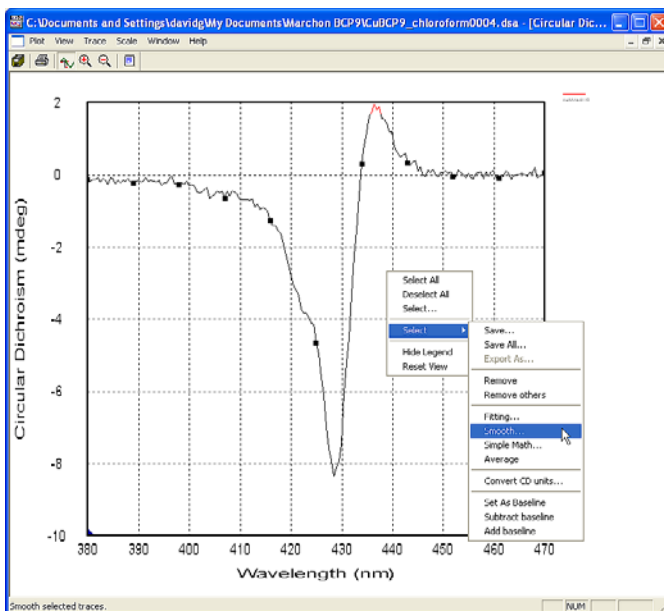


Select and remove the trace which is not baseline-subtracted...



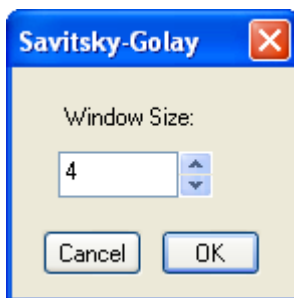
...to leave the baseline-subtracted spectrum only.

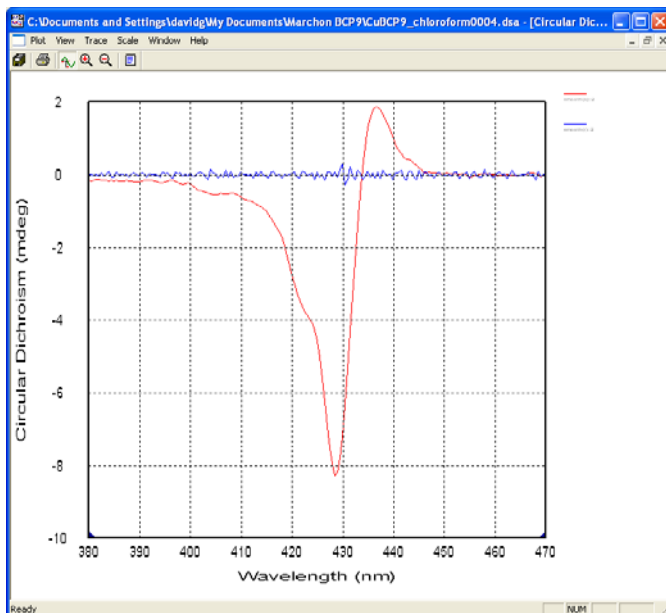
To complete the example manipulation, we will now smooth the baseline-subtracted data. Select this spectrum and choose the *Select* sub-menu *Smooth* option.



Smoothing the background-corrected CD spectrum.

For the conditions under which the CD spectrum in this example was collected, a limited Savitsky-Golay smooth is appropriate – the number of points was set to 4 in the *Smooth* dialogue box. Select the unsmoothed trace and *Remove* it to leave the smoothed spectrum and its residual only (see next page).





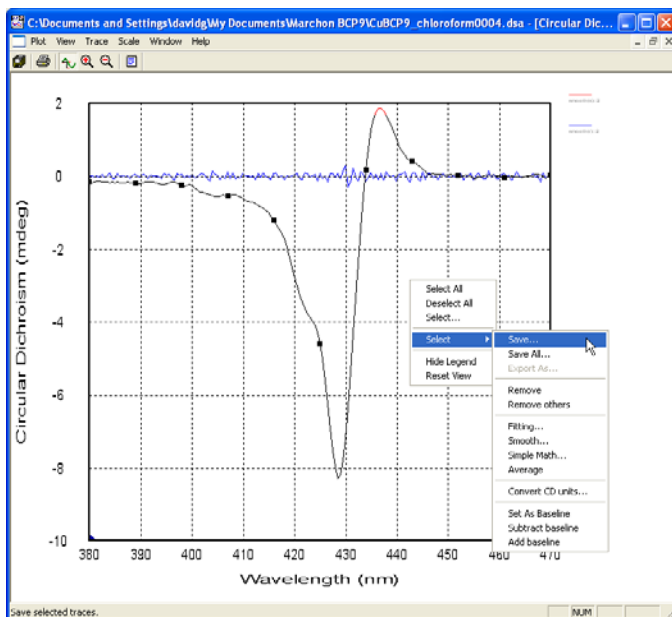
Smoothed spectrum, showing residual randomly distributed about zero.

The data represented in the above figure can be saved as a new file. Note that all original data is left intact and can be restored at any time. Note also that the random distribution of points about zero in residual trace proves that the smoothing has not distorted the data in any way.

NB: It is equally possible to use the alternative method of selection described in Section 2.4.3 (*Select...* and highlight traces from a list of trace names). You will find this latter method more convenient if you are dealing with a number of traces, particularly if they are overlapping.

2.5 Saving data

If you have performed one of the operations in Pro-Data viewer (e.g. smoothing, averaging or baseline subtraction), added comments, or altered the data in any other way you may want to save the data. This can be done using the *Save All* or *Select* sub-menu *Save* option in Pro-Data viewer, as described in Section 2.4.4 and shown below.



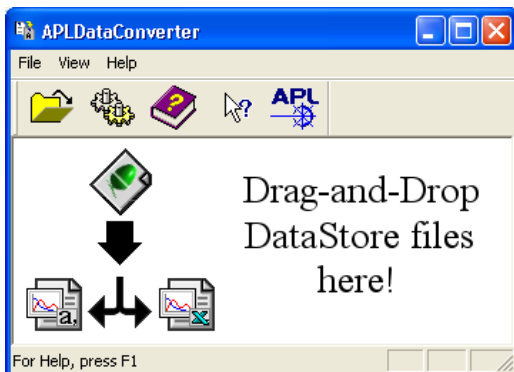
A *Save As* dialogue box will appear and you choose a name and location for the DataStore (.dsa) file.

Alternatively, you can export the data in one of a number of file-formats including a CSV format that is easy to read into any spreadsheet application (such as Microsoft Excel, OpenOffice.org, or Gnumeric). *Export...* is found in the *Plot* menu of the graphical display.

2.5.1 Converting data for use with third-party programs



This icon is installed on your desktop and will start the APL data converter.



The APLDataConverter program has explicit instructions written in the window. In default configuration, it will convert one or more DataStore (Chirascan) files into spreadsheet-friendly comma-separated-variable (.CSV) files that will be written back to the same directory as the original files, which are left intact. The .CSV files can be loaded into any program that accepts the .CSV format (e.g. Excel and Lotus 123). To find out more about the use of the APLDataConverter, run the program and click on



Please note, other file options relate to file formats derived from other APL products.

2.6 Printing

The *Print* icon in Pro-Data viewer will print the current Pro-Data viewer display together with the principal experimental parameters and any comments you have added to the file.

2.7 Operating notes and hints

2.7.1 Selecting Step Size (SS)

The step size will determine the number of data points that make up a spectrum. For a given time per point, larger step sizes will reduce the overall scan time of an experiment but will reduce the resolution of the spectrum; smaller step sizes will improve the resolution of a spectrum but it will take longer to run.

An appropriate step size can be chosen using the equation: $SS = FWHH/20$, where FWHH refers to the full width at half height (in nm) of the narrowest peak in the spectrum.

Guidelines: samples in solution: $0.2\text{nm} < SS < 1\text{nm}$
 gas or vapour samples: $0.1\text{nm} < SS < 0.5\text{nm}$

Specific examples are given in Section 10. *NB* Analysis of protein spectra using the public domain software CDNN requires a 1 nm SS.

2.7.2 Selecting Spectral Bandwidth (SBW)

The spectral bandwidth should be selected to maximise the light reaching the sample, without compromising spectral resolution. For a given signal-to-noise ratio, a narrower bandwidth will take longer to run but will reveal spectral fine structure; a wider bandwidth will permit you to run the spectrum more quickly but may lose detail.

Guidelines: samples in solution: $0.5\text{nm} < SBW < 3\text{nm}$
 gas or vapour samples: $0.1\text{nm} < SBW < 0.5\text{nm}$

Specific examples are given in Section 10.

In the far-UV, increasing the bandwidth can be essential if you have a highly absorbing sample. Doubling the SBW increases fourfold the number of photons reaching the sample. The maximum bandwidth varies with wavelength because of the non-linear dispersion of light by prisms – dispersion increases (and therefore maximum bandwidth

decreases) with decreasing wavelength. The SBW range for the shorter wavelengths are given in the table below.

Wavelength	SBW range
170nm	0.1nm < SBW < 3nm
180nm	0.1nm < SBW < 4nm
200nm	0.1nm < SBW < 6nm

2.7.3 Selecting pathlength and concentration

The pathlength and concentration of a sample should be chosen so the absorbance value at any given point in the spectrum does not exceed 2 absorbance units (a.u). It is good practice to run a simultaneous absorbance scan when performing a CD experiment in order to verify that the absorbance does not significantly exceed this limit.

If the absorbance exceeds the upper recommended limit, you may wish to use a lower pathlength cell or dilute the sample; conversely, if there is insufficient absorbance to generate a reliable CD signal, you may wish to use a longer pathlength cell or increase the concentration of the sample.

2.7.4 Selecting time per point

The time per point is the time taken sampling data for each step. Collected data at each point are averaged to improve the ratio of signal-to-noise. If the noise levels are too high, then more data points will need to be averaged – increase the time per point. This parameter needs to be selected in conjunction with the spectral bandwidth and step size of a scan, as all these factors play an important part in determining the scan time and in optimising spectral quality. Examples of suitable parameters for many common types of experiment have been included in Section 10.

Guidelines: A doubling of the time per point will result in a $\sqrt{2}$ reduction in the noise. A tripling of the time per point will result in a $\sqrt{3}$ reduction in the level of noise, and so on.

2.7.5 Nitrogen purge flow-rate

It is essential that the system be purged with nitrogen constantly whilst the lamp is on. Failure to do this will result in the formation of ozone, which is damaging to health and to the optical components in Chirascan (see Section 2.1).

	N2 flow-rates* (start-up) Lmin ⁻¹	N2 flow-rates (normal) Lmin ⁻¹
Lamp housing	4	1
Monochromator	10	3
Sample chamber	2	1
Total	16	5

It is only when the lamp housing or monochromator seals are broken that the higher flow-rates are required. Unless the instrument has been left idle and unpurged for many days, 10 minutes of purging at the normal flow-rates will be sufficient to establish an oxygen-free atmosphere.

*The maximum flow rate will be determined by the source pressure. If the pressure is significantly below the maximum 6 bar, then it might not be possible to achieve the listed flow rates. If that is the case, set the flow rates to the highest possible, directing the majority of flow through the monochromator.

2.7.6 Measuring protein spectra.

Measuring the CD spectra of proteins is perhaps the most common use to which the Chirascan will be put. In the following paragraphs are some hints and tips on how to ensure that your protein CD spectra are of the highest quality. It is not possible to cover all contingencies but these guidelines will, it is hoped, get you in the right ball-park.

Lower wavelength limit	Solvent	Pathlength	Protein concentration
180nm	H ₂ O, dilute buffer or D ₂ O	□ 0.5mm	0.2 – 0.5 mg/mL
175nm	H ₂ O or D ₂ O	□ 0.2mm	0.5 – 1.0 mg/mL
170nm	D ₂ O	□ 0.1mm	1.0 mg/mL

- 1) Use a short pathlength if measuring into the far-UV. See above table for recommendations.
- 2) Use a bandwidth □ 1nm to ensure the detector is working in a linear regime, especially as you go to the near-UV where samples absorb less light. With the


- sample in place, the detector high-voltage should not drop below 200V. If it does, reduce the bandwidth.
- 3) If you wish to go below 180nm, consider using heavy water – it absorbs less than normal water and is not expensive – buy NMR-standard D₂O from a reputable supplier.
 - 4) If you wish to go to 170nm – and these are very difficult experiments to perform – you will have to employ a cell with a pathlength \approx 0.1mm and use heavy water.
 - 5) Use degassed solvent to prevent bubble formation in the cell (and to remove oxygen).
 - 6) Ensure the instrument is purged properly with nitrogen. Flows of 1L min⁻¹, 3L min⁻¹ and 1L min⁻¹ for the lamp, mono and sample will be perfectly adequate, even for the very short wavelength measurements.
 - 7) It is a good idea to separate the absorbance of cell and solvent from sample. The best way to do this is to measure a baseline with no sample or cell in place, then measure the blank as a sample. That would give you the CD baseline and an absorbance spectrum of the blank. Finally measure your sample. You would then have the CD spectrum of your sample, a baseline to subtract from it, the total absorbance of sample, solvent and cell and the absorbance of solvent and cell only. Subtracting solvent and cell from total will give you the absorbance of the sample only. The reason for doing this is that you will find out that water – or buffer or D₂O – absorbs very strongly as you approach and go below 180nm. It is important that you keep the total absorbance within reasonable bounds. If you keep your total absorbance below 2AU, you should be OK.
 - 8) Remember that photodegradation of your sample will occur if you use an unnecessarily high intensity beam.

2.8 Example spectra.

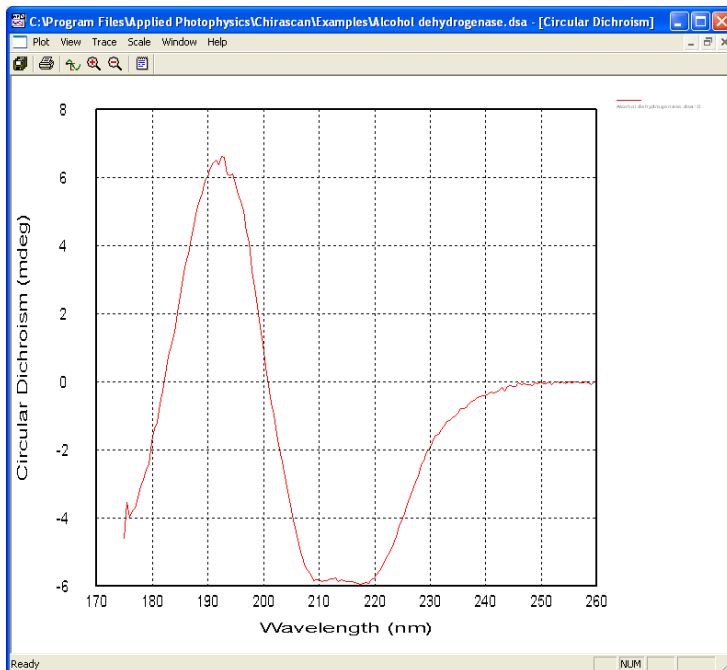
There are several files installed on the hard disk that can be used as templates to set up the Pro-Data interface or as didactic tools to familiarise oneself with the use of Pro-Data viewer. The files are held in

C:\Program Files\Applied Photophysics\Chirascan\Examples



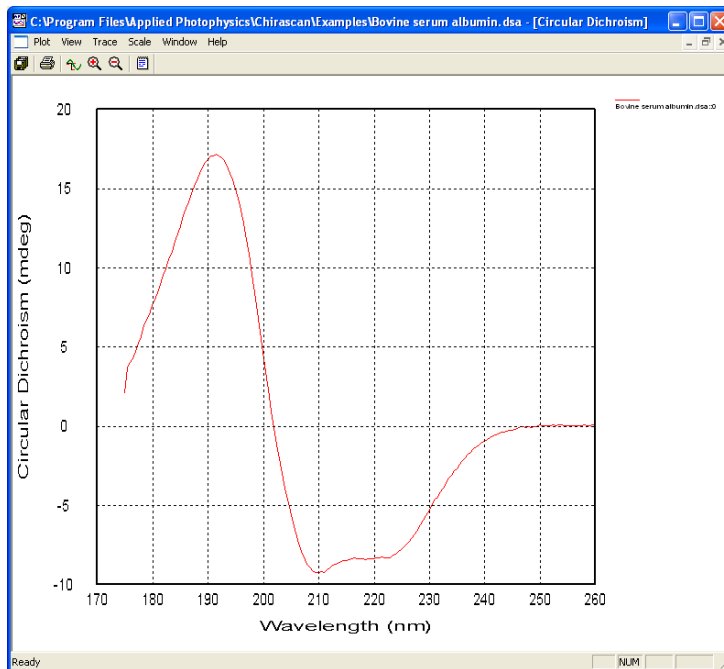
and can be accessed using the  icon from the Pro-Data interface toolbar and in Pro-Data viewer by navigating to the directory in the launchpad.

2.8.1 Alcohol dehydrogenase



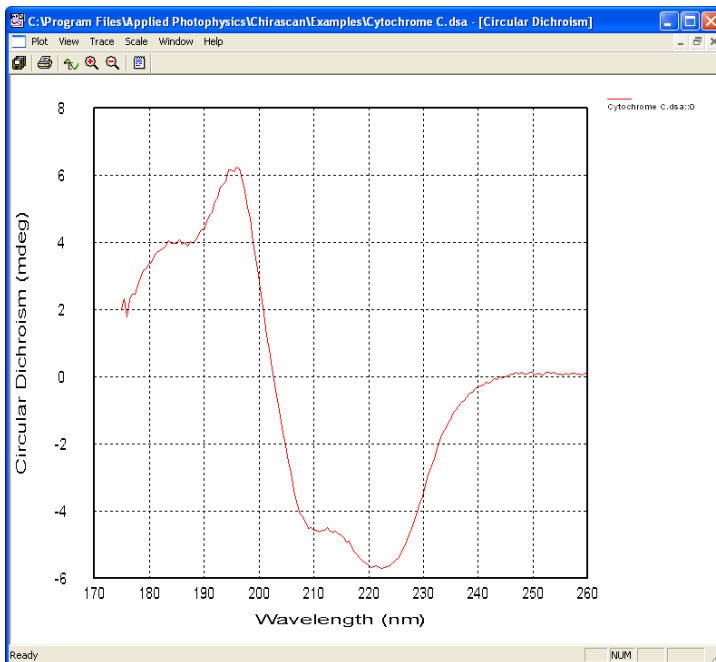
Scan range	260 – 175 nm
Spectral bandwidth	1 nm
Step size	0.5 nm
Pathlength	0.1 mm
Concentration	1 mg/mL
Solvent	Water
Time per point	3 s
Total N2 flow	5 Lmin-1

2.8.2 Bovine serum albumin



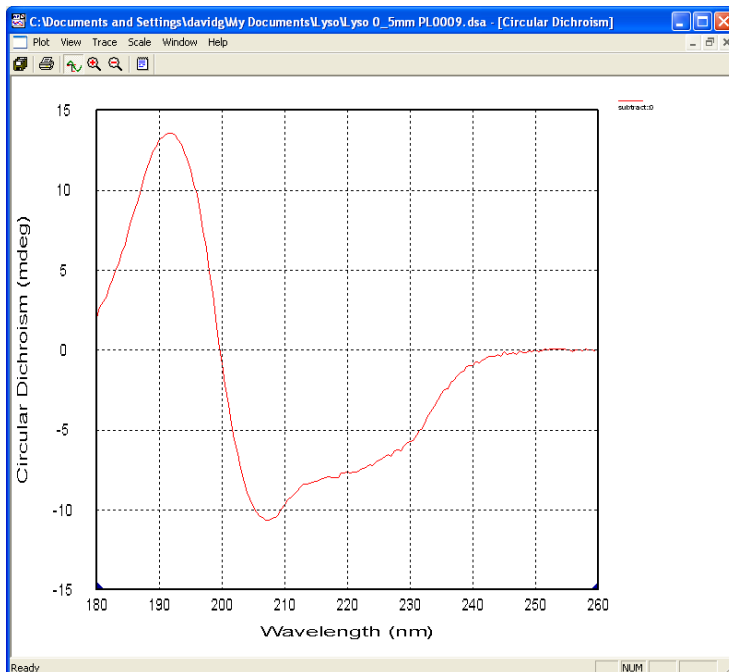
Scan range	260 – 175 nm
Spectral bandwidth	1 nm
Step size	0.5 nm
Pathlength	0.1 mm
Concentration	1 mg/mL
Solvent	Water
Time per point	3 s
Total N2 flow	5 Lmin-1

2.8.3 Cytochrome C



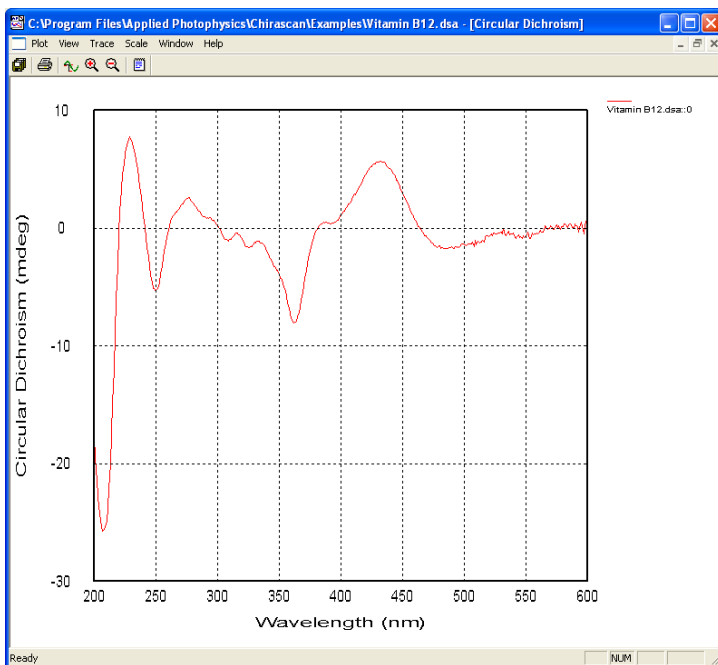
Scan range	260 – 175 nm
Spectral bandwidth	1 nm
Step size	0.5 nm
Pathlength	0.1 mm
Concentration	1 mg/mL
Solvent	Water
Time per point	3 s
Total N2 flow	5 Lmin-1

2.8.4 Lysozyme



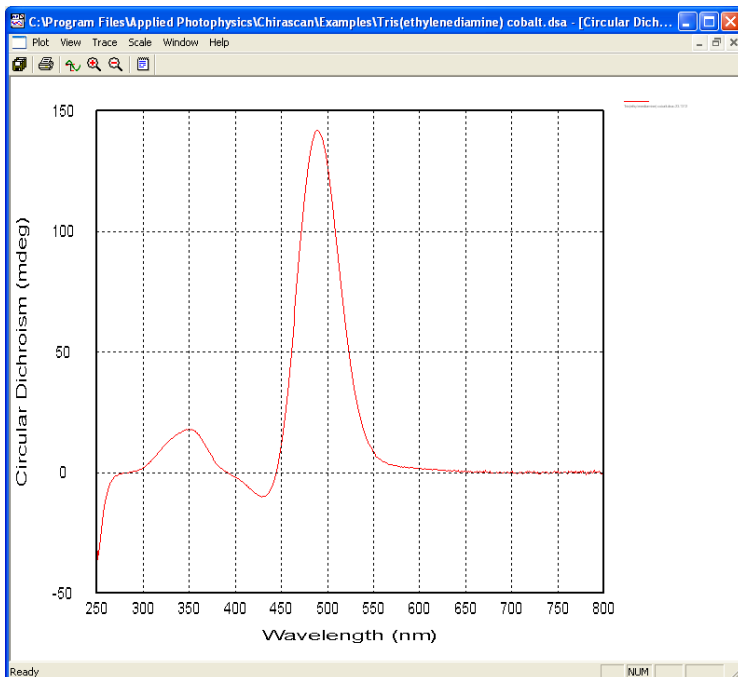
Scan range	260 – 185 nm
Spectral bandwidth	2 nm
Step size	0.5 nm
Pathlength	0.5 mm
Concentration	0.2 mg/mL
Solvent	Water
Time per point	1s, 3-scan average
Total N2 flow	5 Lmin-1

2.8.5 Vitamin B12



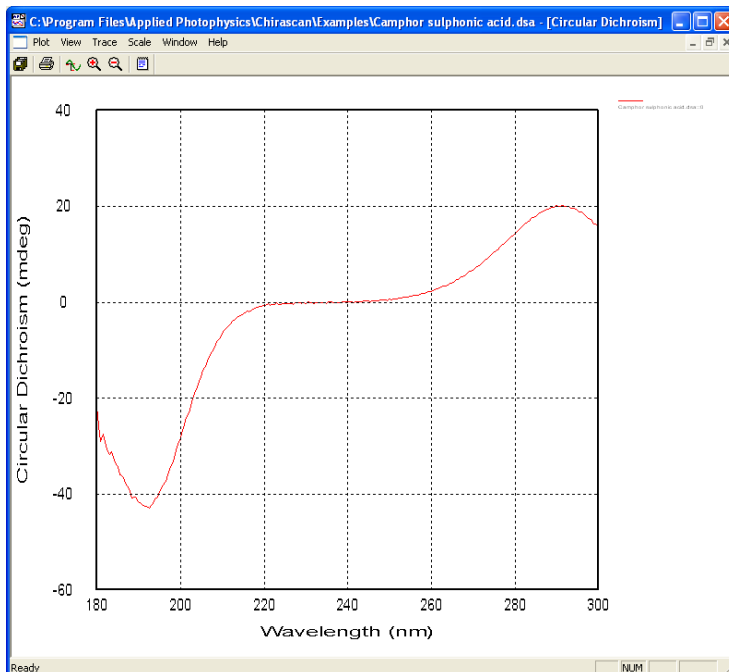
Scan range	600 - 200 nm
Spectral bandwidth	1 nm
Step size	1 nm
Pathlength	1 mm
Concentration	1 mg/mL
Solvent	Water
Time per point	2 s
Total N2 flow	5 Lmin-1

2.8.6 Tris(ethylenediamine) cobalt chloride



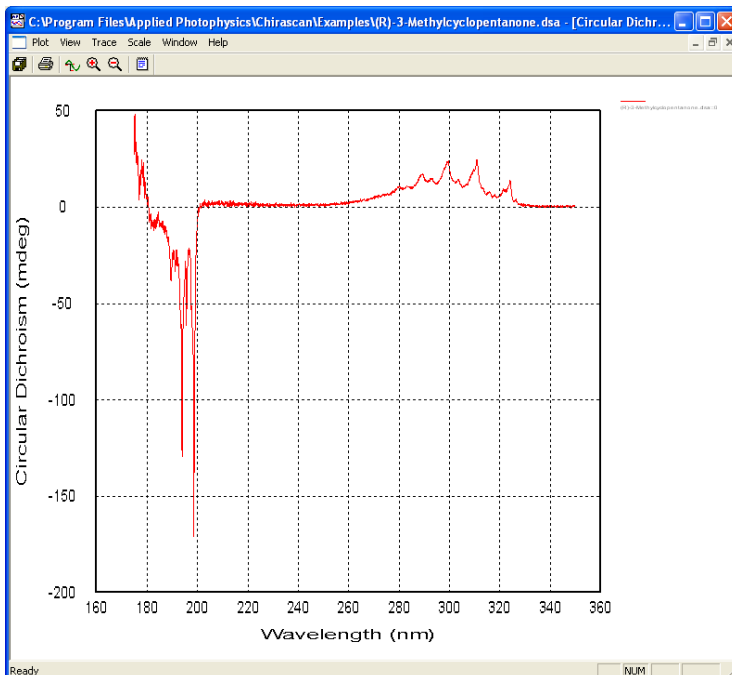
Scan range	800 - 250 nm
Spectral bandwidth	1 nm
Step size	1 nm
Pathlength	10 mm
Concentration	1 mg/mL
Solvent	Water
Time per point	0.5 s
Total N2 flow	5 Lmin-1

2.8.7 Camphor sulphonic acid



Scan range	300 - 180 nm
Spectral bandwidth	0.5 nm
Step size	0.5 nm
Pathlength	1 mm
Concentration	0.6 mg/mL
Solvent	Water
Time per point	1.75 s
Total N2 flow	5 Lmin-1

2.8.8 (R)-3-methylcyclopentanone



Scan range	350 - 175 nm
Spectral bandwidth	0.1 nm
Step size	0.1 nm
Pathlength	10 mm
Concentration	Vapour
Solvent	-
Time per point	1 s
Total N2 flow	5 Lmin-1

2.9 Troubleshooting

If the instrument does not operate properly, please use the following table to eliminate trivial causes of incorrect function. There is also a 'frequently asked questions' section on the website (<http://www.photophysics.com/faqch.php>) to which you should refer if the table below does not help. If the symptoms persist, please contact Applied Photophysics Technical Support (contact details at front of manual) with a detailed description of your difficulty and the name of your spectrometer. Each spectrometer has a name that can be found at the front of the manual and on the front of the monochromator.

Symptom	Cause	Corrective action
Instrument / computer cannot be switched on.	No power available.	Check that all power cables are correctly plugged in and that the power at the mains outlet is switched on.
		Check that all supply fuses are intact.
No detector signal.	No light - xenon lamp is not ignited.	Put the rocker switch in the 'I' (on) position and press the red ignite button.
	No light - shutter is in the closed position.	Use the Ptro-Data interface to <i>Open</i> the shutter.
	No light – slits too narrow or closed.	Check the bandwidth in the Monochromator section of the Pro-Data interface and set the bandwidth to a reasonable value, e.g. 1nm
	No HV on the detector.	In this case, light might be reaching the detector but without any high voltage applied to the detector there will be no signal. Check the detector cable is correctly connected and then use the Pro-Data interface to <i>Restore</i> the HV.
	No light – sample is opaque.	Reduce concentration and / or pathlength of sample and / or buffer.
	No light in far-UV – instrument is not purged.	Oxygen absorbs light at wavelengths below 190nm. Ensure that the purge tubes are connected at both ends, that there is nitrogen available and purge the instrument with clean nitrogen.
Signal is too noisy.	Bandwidth too narrow	Check the bandwidth in the mono section of the Pro-Data interface and set it to a reasonable value, e.g. 1nm.
	Sample or buffer is highly absorbing.	Reduce concentration and / or pathlength of sample and / or buffer.
	Lamp is old.	If the lamp has been run for more than 1,000 hours, its output, especially in the far-UV, will be low. Replace lamp.
	Unstable line voltage	Use stabilised supply.
	Electromagnetic interference from nearby equipment.	Remove the source of noise from the proximity of the spectrometer.

2.10 Notes

Use these blank pages for your own notes.

