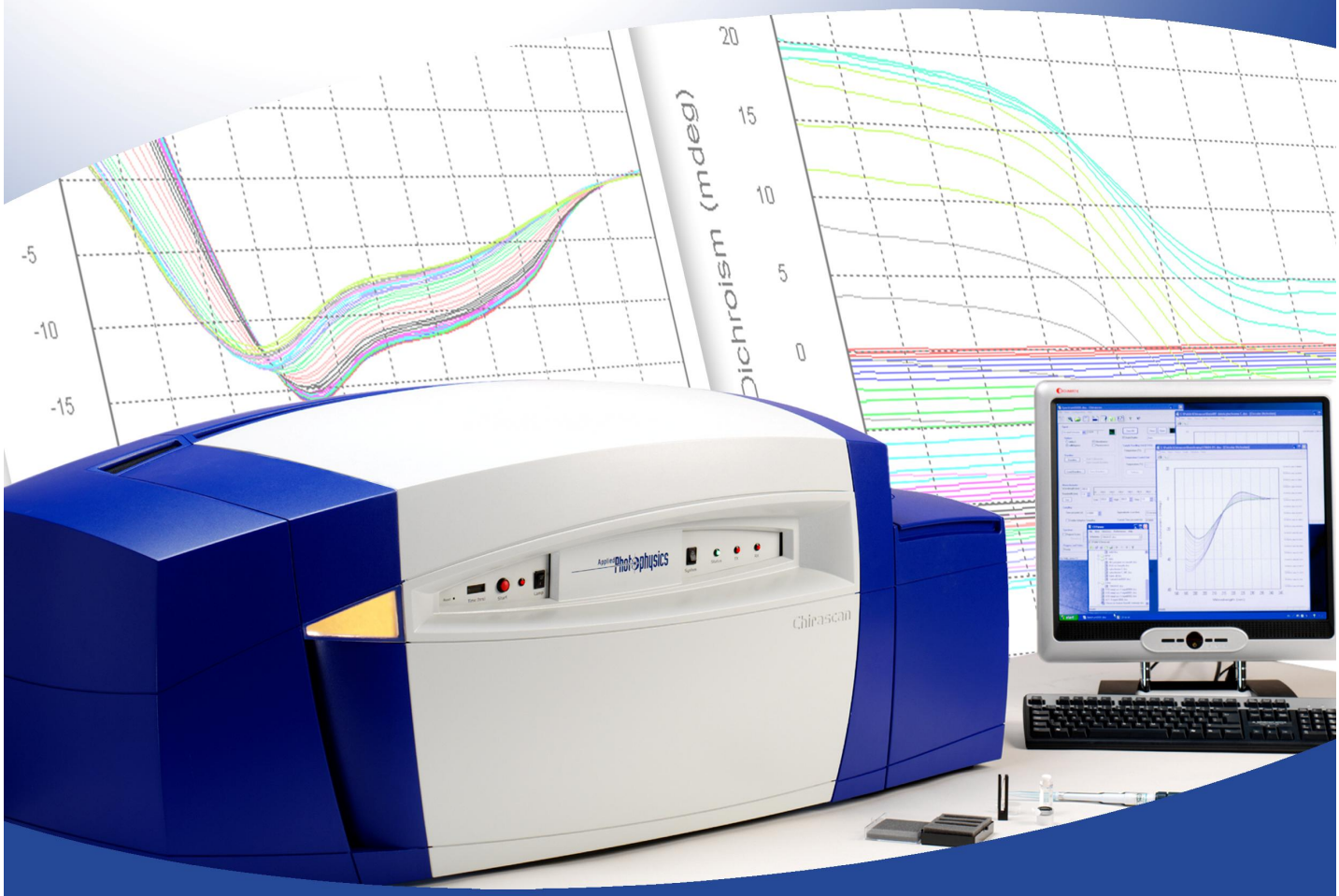
AppliedPhotophysics

Chirascan™ & Chirascan™-plus CD spectrometers

Sets a new standard for steady-state circular dichroism spectroscopy



- Unrivalled far-UV performance
- Low running costs
- Full range of accessories including integrated stopped-flow

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Chirascan / Chirascan-plus CD Spectrometer

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Introduction

Applied Photophysics has pioneered development of CD instrumentation over recent years. Starting in 1994, with the release of the world's first production CD stopped-flow (the CD.1C and CD.2C accessories for our SX-series of stopped-flow spectrometers), this was followed in 1999 with the first dedicated CD stopped-flow spectrometer, Pi-Star 180, which was also designed for steady-state CD spectrometry.

Chirascan is the result of over 3 years research and development (drawing also on information gathered from over 300 scientists in the field of CD spectrometry) and offers truly world beating CD performance in terms of speed, sensitivity and accuracy.

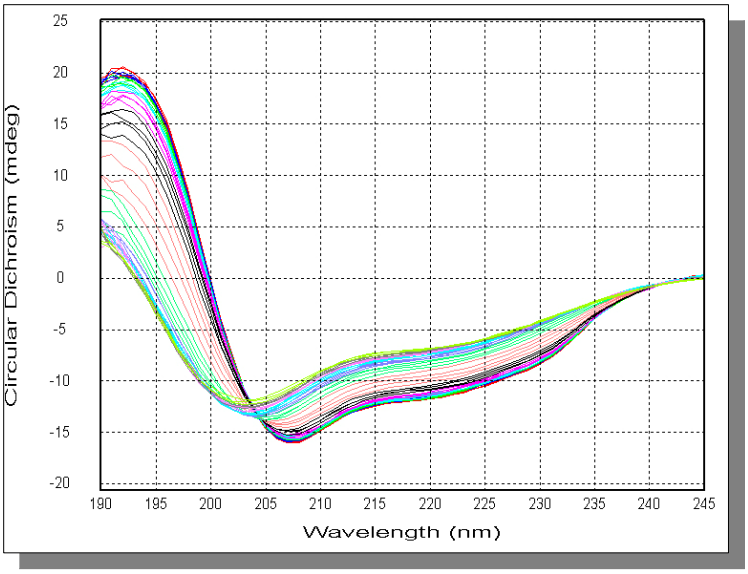
Chirascan sets new standards for steady-state circular dichroism spectroscopy. It incorporates innovative optical design features to maximise light throughput, particularly in the far-UV wavelength region, and a sophisticated digital data acquisition system that facilitates the rapid collection of more accurate and precise CD spectra. Unlike conventional CD spectrometers which use analogue electronic filters which irreversibly smooth (and potentially distort) CD spectra during acquisition by the use of analogue filters, Chirascan's digital approach ensures that unmodified CD spectra are collected and any post-acquisition smoothing of the CD spectra will be non-distorting and completely reversible. This approach also simplifies the operation – Chirascan is as straightforward to use as a single-beam spectrophotometer.

A large range of accessories are available, ensuring the researcher can be confident of a highly effective and future-proof spectrometer that can be adapted as research interests evolve.

Chirascan Standard Features

- **Exceptionally high sensitivity. Innovative optical design provides vastly superior light throughput compared with older designs, resulting in faster, lower-noise CD spectra**

- **Able to collect thermal denaturation CD spectra in a single experiment; enabling identification of the secondary structural changes associated with each phase transition**

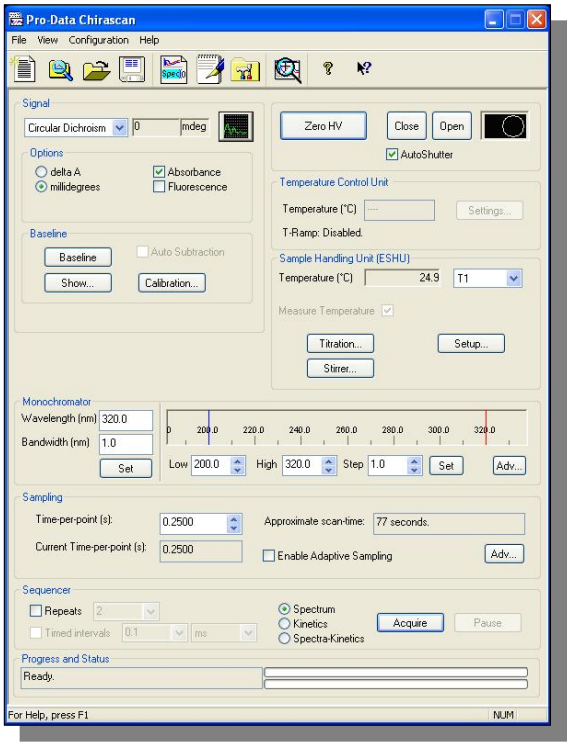


- **Digital acquisition (no electronic filtering) guarantees data fidelity and avoids the risk of distortion associated with the use of electronic time constants. This also simplifies operation**

- **5 detection channels: CD, Absorbance/Transmission, HT, Temperature and Voltage. Simultaneous multi-channel data acquisition ensures that all key information is recorded with every measurement you make**

- **Very low nitrogen. Rapid and efficient nitrogen purging combined with a sealed monochromator housing ensures that just 5 l/min^{-1} is required for far-UV work**

- **Moveable detector. The detector position is easily adjustable and can be set close to the cell to optimised performance with highly scattering samples e.g. membrane proteins**



- **Pro-Data control software with comprehensive acquisition, display and analysis tools including: live display, advanced monochromator settings, kinetic acquisition and analysis, post-acquisition smoothing, export to spreadsheet and other file formats etc.**

- **Pro-Data viewer software can be installed on an unlimited number of PC's for off-line data inspection and analysis. An emulation version of the Pro-Data instrument control software can be also installed for off-line training**

- **CDNN secondary structure analysis software**

- **24 month warranty**

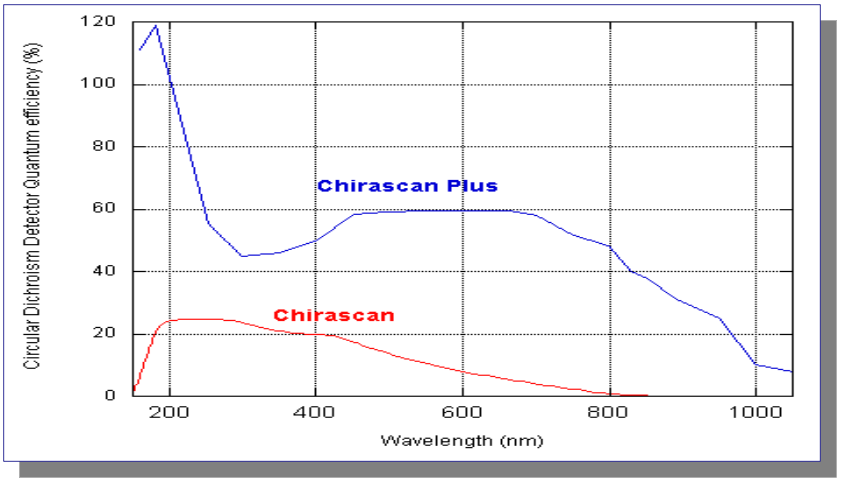
- **21 CFR Part 11 compliance is available as an option**

- **Straightforward upgrade to Chirascan-plus**

Chirascan-plus Features

Chirascan-plus is an enhancement of the standard Chirascan instrument which offers truly remarkable speed, sensitivity and flexibility. Chirascan-plus builds on Chirascan's high light throughput and use of digital electronics with the introduction of a new, high performance, solid state detector. Chirascan-plus offers:

- **Improved sensitivity. The high quantum efficiency of the solid state detector in comparison with the standard photomultiplier detector (see figure right) leads directly to improved signal-to-noise over the standard Chirascan. For example in the region of 180-260 nm, a common range for many CD applications, there is a 2-fold increase in signal-to-noise. This means that similar data quality can be achieved with a 4-fold increase in scanning speed**



- **Wider wavelength range: 165–1100nm (c.f. 165-900nm with the standard Chirascan). The signal-to-noise advantage increases significantly at visible wavelengths and into the NIR**
- **More accurate absorbance measurement. Chirascan-plus detection electronics have a known absolute gain, consequently the absorbance measurements, recorded simultaneously with the CD, are as accurately as a high quality single beam spectrophotometer.**
- **Flatter baselines. A significant contributor to baseline offsets in CD spectrophotometers is the birefringence of the photomultiplier window itself. The solid-state detector does not have a window and so does not contribute to the baseline.**
- **Insensitive to stray magnetic fields. The solid state detector is impervious to magnetic fields up to 5 Tesla. This makes the detector ideal for specialist techniques such as Magnetic CD**

Pharmaceutical Applications - Chirascan-plus DMS

Whilst the signal-to-noise advantage will benefit demanding techniques such as CD stopped-flow, Chirascan-plus also offers a whole new approach to experimental design based on high data content, fast data acquisition and low sample volumes. Chirascan-plus dynamic multi-mode spectroscopy (DMS) is a package designed principally for the pharmaceutical industry for collecting and globally analysing thermal denaturation CD, absorbance and fluorescence spectra collected in a single thermal denaturation experiment. Chirascan-plus DMS is described in more detail in a separate document.

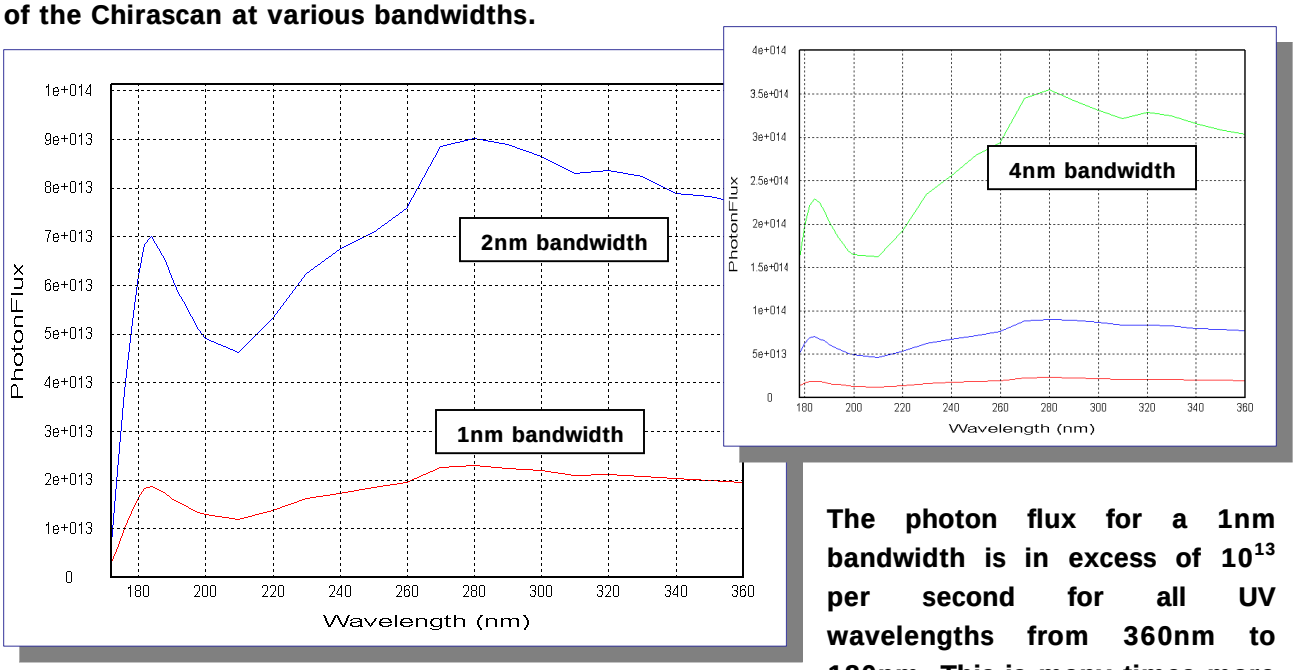
Performance Specifications: Chirascan and Chirascan Plus

Light Source:	***150W air cooled Xe lamp***	
Monochromator:	***F/7 split-Wollaston prism, dual-polarising, dual-dispersive Optics; wavelength limits 160 – 1360nm***	
Wavelength Accuracy:	***±0.2nm (170nm – 400nm), ±0.5nm (> 400nm)***	
Wavelength Precision:	***±0.05nm (170nm – 400nm), ±0.1nm (> 400nm)***	
Wavelength Resolution:	***0.1nm at all wavelengths***	
Slit Bandwidth:	***0 to 2nm @ 160nm (in 0.1nm increments) 0 to 4nm @ 180nm (in 0.1nm increments) 0 to 7.5nm @ 200nm (in 0.1nm increments) 0 to 15nm @ 234nm etc.***	
Stray Light:	***< 3 ppm at 200nm***	
Baseline Stability:	***< 0.02m°/hour***	
CD & absorption detector:	***High-performance UV-Vis photomultiplier tube***	
Wavelength range:	***Practical limits with sample in place: 170nm – 900nm Optional IR-extended detector for wavelengths > 900nm Practical limits with sample in place: 170nm – 1150nm***	
CD RMS Noise: (All measurements at 1nm BW and 2s integration time)	***0.07m° @ 175nm 0.03m° @ 180nm 0.02m° @ 185nm 0.03m° @ 200nm 0.03m° @ 250nm 0.04m° @ 500nm 0.27m° @ 800nm - @ 1000nm***	***0.03m° @ 175nm 0.02m° @ 180nm 0.01m° @ 185nm 0.02m° @ 200nm 0.02m° @ 250nm 0.02m° @ 500nm 0.04m° @ 800nm 0.05m° @ 1000nm***
Wavelength scanning:	***Constant sampling & adaptive sampling stepped-scan***	
Kinetic mode:	***Linear, logarithmic and split time-base***	
CD scale and resolution:	***±6000m° with automatic scaling. Resolution better than 0.001m° in 6000m°***	
Standard detection modes		
Spectroscopic probes:	***Simultaneous circular dichroism and absorption Configurable for FDCD (as standard) and fluorescence***	
Other probes:	***Simultaneous temperature, detector HT, DC target voltage***	
Nitrogen Purge Requirements:	***5 l/min nitrogen (all wavelengths). 2 l/min nitrogen (above 200nm)***	
Startup time:	***15mins***	
Software included:	***Pro-Data control & viewer software, CDNN secondary structure analysis software, APL data converter.***	
PC interface:	***Windows XP Professional***	
Electrical Requirements:	***220/240, 110 V. 440 VA***	
Available options:	***Single-cell and multi-cell peltier, titrator, magnetic CD, fluorescence detection, fluorescence scanning monochromator, NIR detection, low temperature cryostat, stopped-flow, ORD and linear dichroism detection, FDA: 21 CFR Part 11 Compliance***	

Performance Notes

Quality of measurement and its relation to light throughput

Chirascan's high light throughput, particularly at far-UV wavelengths, is the key reason for its superior sensitivity and speed. Shown below are calibrated radiometric scans of the light flux of the Chirascan at various bandwidths.



The photon flux for a 1nm bandwidth is in excess of 10^{13} per second for all UV wavelengths from 360nm to 180nm. This is many times more intense than the flux of any other commercially available CD spectrometer. Should more light be required, a bandwidth of up to 4nm can be maintained down to 178nm, a feature that is unique amongst prism-based CD spectrometers and derives directly from the innovative optical design of Chirascan. It means that for Chirascan, where the photon flux at a 1nm bandwidth is vastly superior to that of other CD spectrometers, there is more than a further order of magnitude of light flux in reserve.

The superior light throughput of Chirascan translates directly to superior data quality in terms of sensitivity and/or speed. Here are a few examples of why this is important:

- **In laboratories where there is high demand on the instrument, productivity is significantly increased without compromising data quality**
- **More data can be generated in a single experiment – for example; far-UV wavelength-scanning in combination with continuous-ramp thermal melts generate a complete picture of protein secondary structure denaturation in a single denaturation experiment taking approximately one hour**
- **In experiments that are demanding of sample, for example CD stopped-flow, the number of repeat measurements (and hence the volume of sample) required to achieve acceptable quality is greatly reduced – see page 23.**

Advantages of Stepped Scanning

Chirascan-plus acquires CD spectra using stepped scans. The CD signal is sampled at discrete wavelength points in the spectrum for a time defined by the user. Each measured point is the average of tens of thousands of samples and the number of points in the CD spectrum (or the wavelength increment) is determined by the user.

There are very good reasons for using stepped scans:

- **Each CD measurement in the spectrum is accurate because it is sampled at a single point with the monochromator held stationary**
- **Each CD measurement has an associated error and therefore has scientific validity**
- **No electronic filtering (time-constant filtering) is used to smooth and potentially distort the CD spectrum**

Because Chirascan has very high light throughput (see above), the raw, unsmoothed spectrum is of high quality and in general will need no further treatment. However, post-acquisition smoothing tools can be used to remove random noise elements if required.

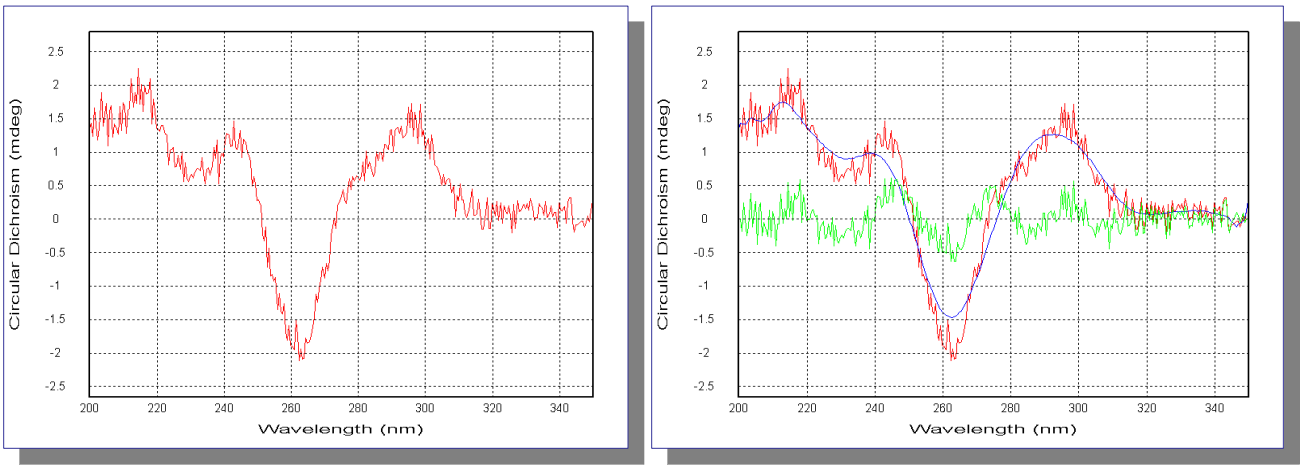
Two key advantages of post-acquisition smoothing are:

- **A residual plot can be generated and inspection will show whether or not the spectrum has been distorted by the smoothing process**
- **The smoothing process is fully reversible and the original (raw) data are never lost**

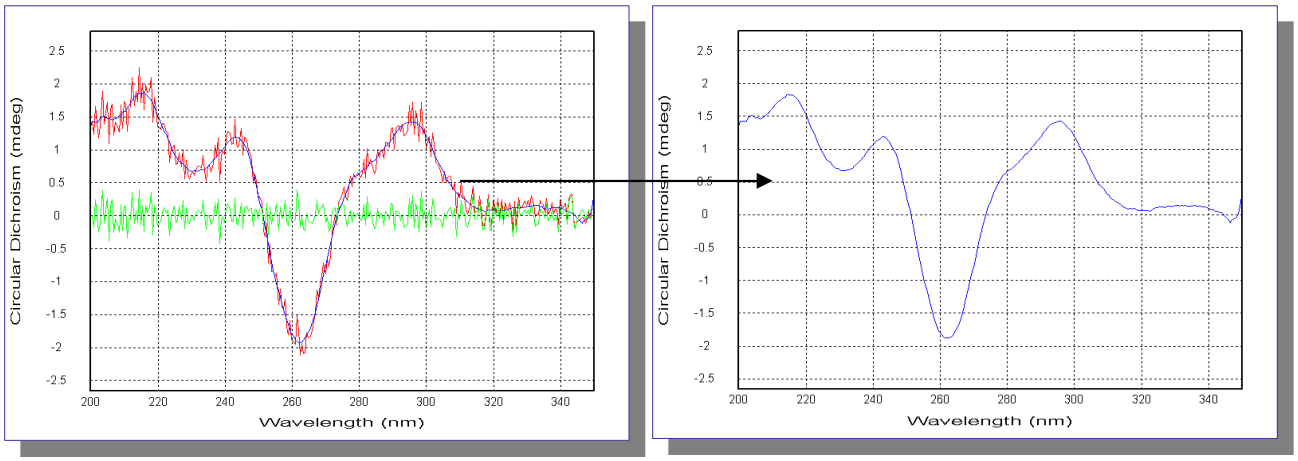
Of course data can be over-smoothed even when using post-acquisition filtering techniques (see section below) but this is always evident and can be corrected.

It should be noted that for continuous scanning, none of the above is true: measurements are made with the monochromator in motion; time constants are applied which damp the signal and disguise the true quality of the spectrum; it is impossible to calculate the error in a measurement; smoothing is carried out during data acquisition and may distort the spectrum; electronic smoothing it is not reversible. Continuous scans can appear to have low noise but, because the data are smoothed during the acquisition, the user cannot assess the true quality of the CD spectrum or whether the smoothing process has distorted its shape. There is no valid reason for collecting data in this way: it disguises poor data quality and can distort spectral features. When comparing the performance of different CD spectrometers, it is essential to compare the stepped scan data in order to make a valid comparison.

Post-Acquisition Smoothing

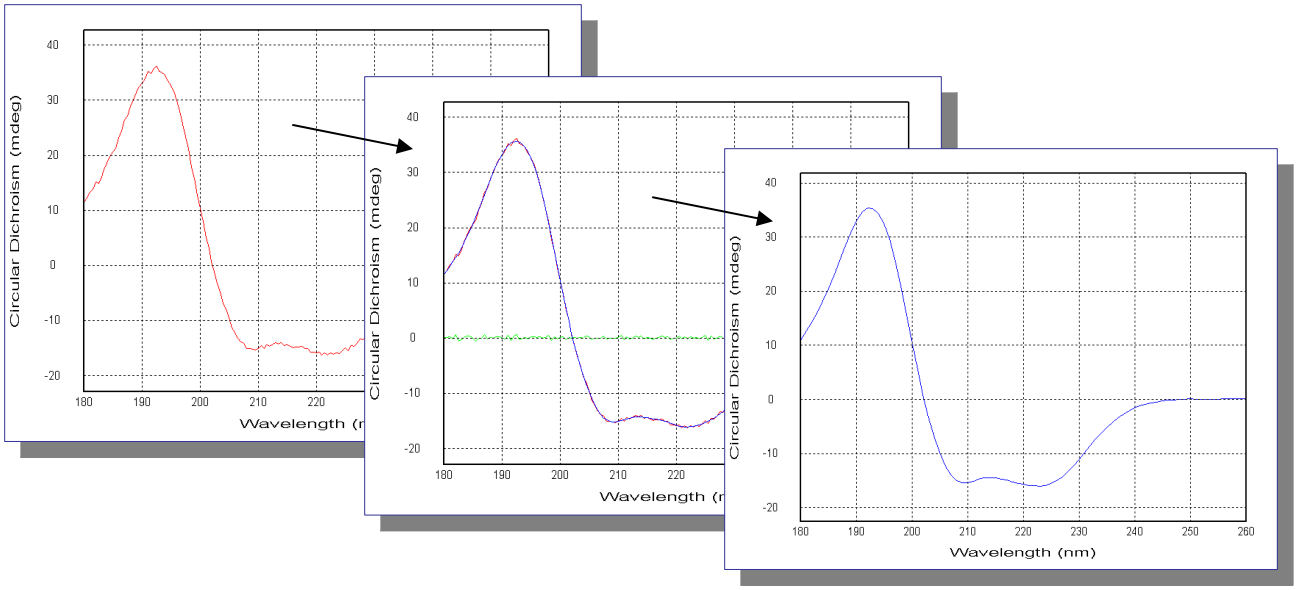


The CD spectrum shown above (left) was recorded on Chirascan and is raw (unsmoothed) data. In this example the spectrum is fairly noisy primarily because of the small size of the CD signal and high scan speed. If we now *over*-smooth this spectrum (as shown above right), the resulting spectrum (blue trace) appears very smooth but the distortion is immediately obvious simply by comparing it with the raw spectrum (red trace). The distortion can also be seen from the residual spectrum (green trace) which is non-random about the x-axis.



A valid smooth of the same spectrum (above left) shows no distortion and, as can be seen from the residual trace, only random noise has been subtracted from the data.

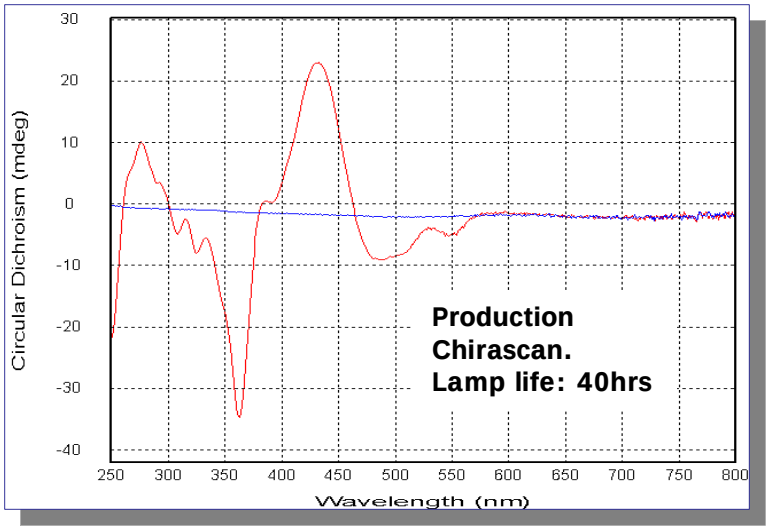
The second example (below) is more typical. The unsmoothed spectrum was acquired in 38 seconds and is of high quality due to Chirascan's high light throughput in the far-UV wavelength region. However some post-acquisition smoothing may be desirable to remove random noise elements.

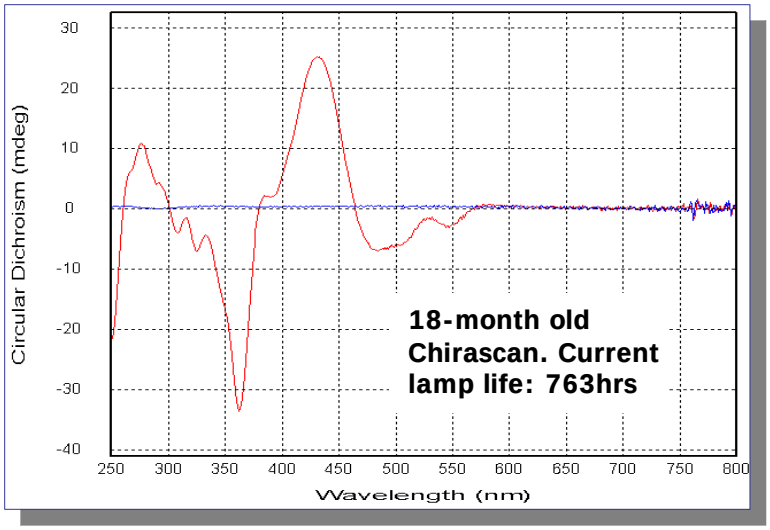


In summary, continuous scans use electronic filters to generate smooth CD spectra but may also produce distortion of the CD spectra. Stepped-scans record unfiltered CD measurements and so are always undistorted. If required, post-acquisition tools can be applied to smooth these spectra in a controlled and reversible way.

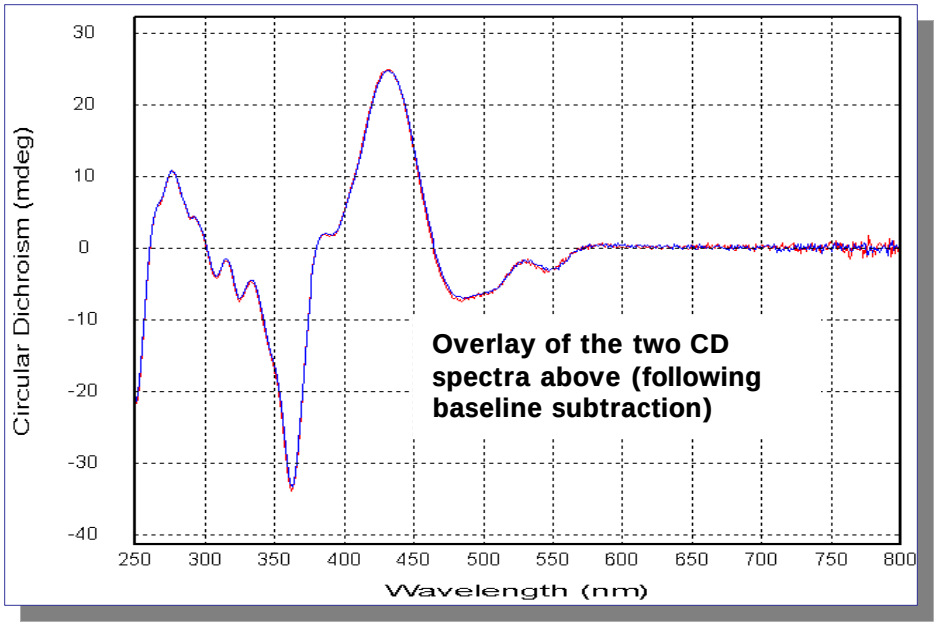
Long Term Stability and Accuracy

Long term stability is of key importance for any CD spectrometer as it will be expected to be in service for many years. The adjacent spectra show the CD spectra of Vitamin B12 recorded on two Chirascan spectrometers: a new instrument ready to leave the factory and an 18 month old instrument with a lamp that is nearing the end of its recommended life. The spectra shown are raw (unsmoothed) data collected back-to-back using the same sample and under the conditions tabulated below. The corresponding baseline for each instrument is overlaid (blue spectrum).





Sample / concentration	Vitamin B12 / 0.2mg/mL
Wavelength range	800nm – 250nm
Bandwidth	1nm
Step size	1nm
Duration of each scan	~ 10 minutes
Pathlength	5mm



Following baseline subtraction, these CD spectra are overlaid for comparison in the figure (left). As can be seen, the spectra are virtually identical, underlining the long term accuracy and stability of Chirascan and Chirascan-plus, and the long lifetime of the lamp.

Low Nitrogen Usage

It is essential that CD spectrometers are purged with a steady stream of nitrogen gas in order to prevent generation of ozone by the xenon arc source and remove oxygen from the light path (oxygen will absorb light at wavelengths in the far-UV)

Chirascan requires a total nitrogen flow of only 5 litres/min when working in the far-UV wavelength region and only 2 litres/min when working above 200nm.

Chirascan's sealed design ensures that even when it has been unused (and unpurged) for several days, the start-up time to achieve a good nitrogen environment for far-UV CD measurements is still rapid.

For the purpose of efficient nitrogen purging Chirascan has three separate nitrogen inlets, each with its own flow-meter:

- **a sealed lamp housing: requiring 1 l/min**
- **a sealed monochromator: requiring 3 l/min (or 1 l/min when working >200nm)**
- **a sealed light path within the sample chamber: requiring 1 l/min (or zero purge >200nm)**

The sealed light path within the sample chamber ensures that the sample chamber can be opened, and the sample cell removed, without compromising the nitrogen environment in the region of the cell holder. Hence the user does not have to wait for the nitrogen environment to be re-established after changing the sample under investigation. This also enables the routine measurement of absorbance spectra with CD spectra because good absorbance measurements depend on a comparison of the detector voltage with and without the sample under identical nitrogen conditions.

Long Lamp Life

It is recommended that the xenon lamp is replaced every 1000 hours. The current lamp life is recorded automatically monitored and displayed by the lamp ignition switch.

Software Upgrades, Licences and Access

Relevant software upgrades are always available for the lifetime of the instrument and are free of charge.

The Pro-Data Viewer software (for data display, manipulation and analysis) can be installed on an unlimited number of PC's for off-line data inspection.

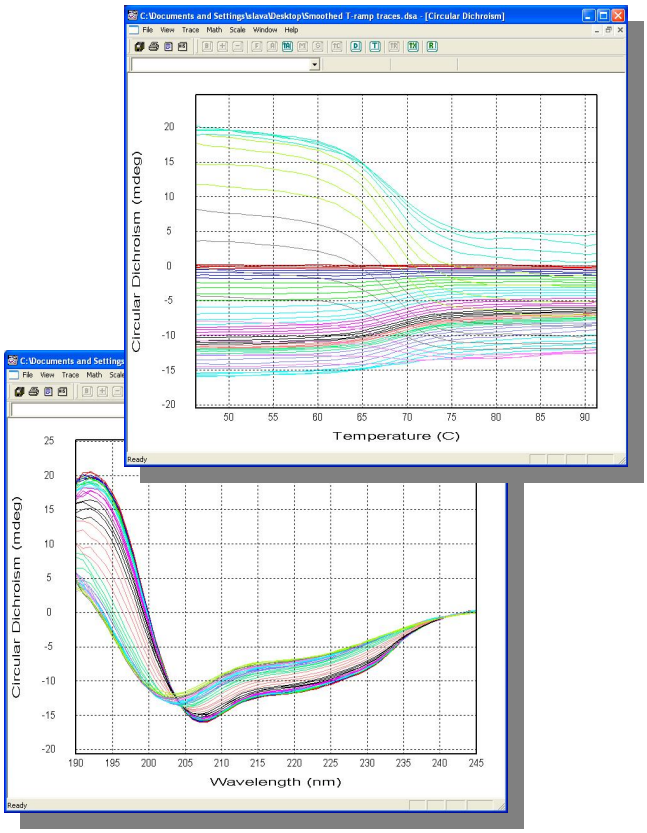
An emulator version of Pro-Data Chirascan (instrument control software) can also be installed on an unlimited number of PC's allowing users to gain familiarity with the instrument control at their desktop.

Upgrade Options for new and existing systems

PCS.3 Single Cell Peltier Temperature Controller

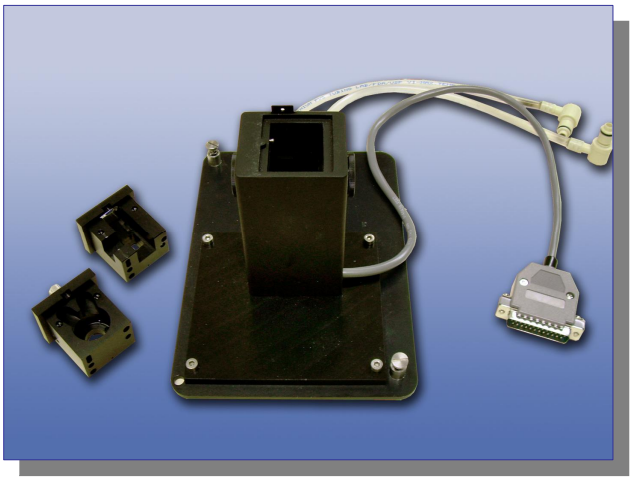
Chirascan can be equipped with an optional peltier temperature controller for rapid and precise temperature control of the sample cell. Chirascan's Pro-Data control software enables extensive temperature and ramping control for standard CD, absorbance and fluorescence signal measurements.

This PCS.3 is based around the Quantum North West TC125 Unit. The unit features a wide operating temperature range (-40°C to 105°C using a circulating chiller unit), excellent thermal contact and accommodates both rectangular and cylindrical and cuvettes. A full list of features is shown below.



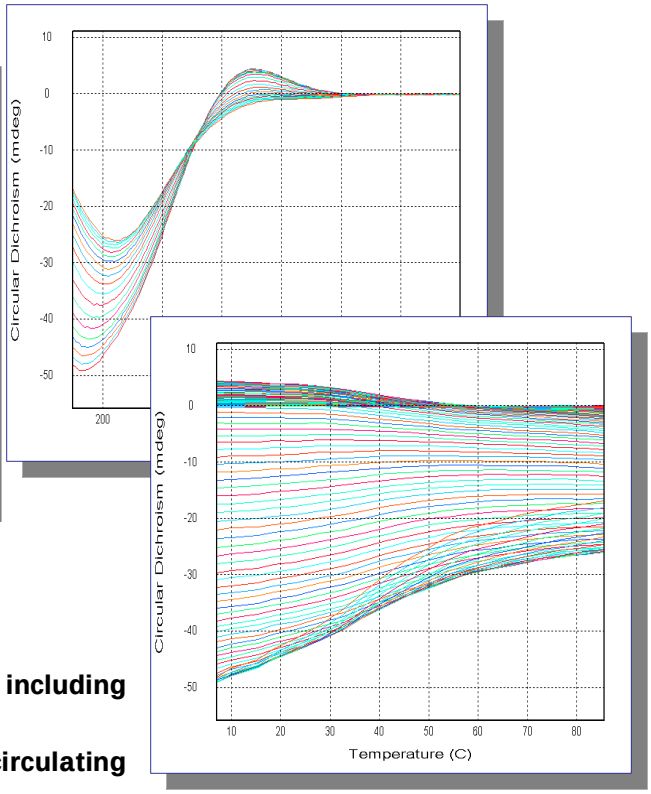
Accessory Features

- **Temperature range -40°C to 105°C (with circulating chiller unit)**
- **Temperature regulation precision: +/- 0.02°C**
- **Temperature slew rate 10°C per minute**
- **Magnetic stirring as standard**
- **Cuvette temperature probe for monitoring sample temperature as standard**
- **Optional cylindrical cuvette-holder available**
- **Total hardware control via Chirascan software**
- **Extensive step-wise and continuous temperature ramping experimental options via Chirascan software**
- **Simple mounting in the sample handling unit**
- **Compatible with all Chirascan signal acquisition mode options**



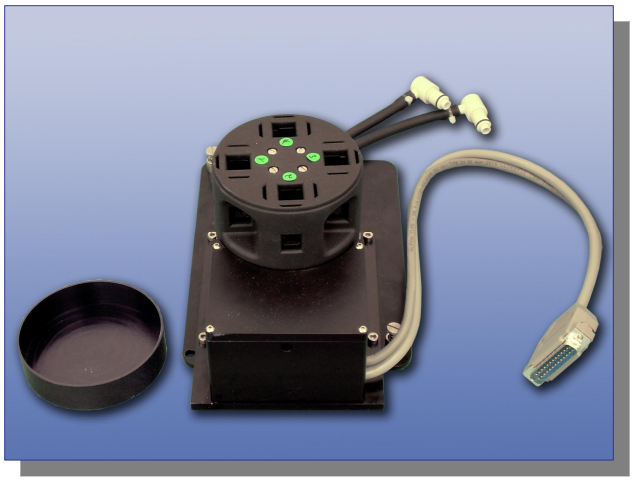
PCM.4 Four-Cell Auto-Changer with Peltier Temperature Controller

Chirascan can be fitted with a 4-cell peltier temperature controller for rapid and precise temperature regulation of up to four samples. The PCM.3's turret design ensures that it is also suitable for fluorescence detection (and simultaneous CD and fluorescence detection). Chirascan's Pro-Data software provides full control, whether for single temperature, temperature-dependant spectra or ramping temperature measurements. The accessory comprises an external temperature controller (Quantum North West TC 401 Unit) and the Chirascan four-cell carousel with variable speed magnetic stirrer capability in all cell positions as standard. The standard operating range of the QNW temperature controller is -40°C to 110°C using a circulating chiller unit.



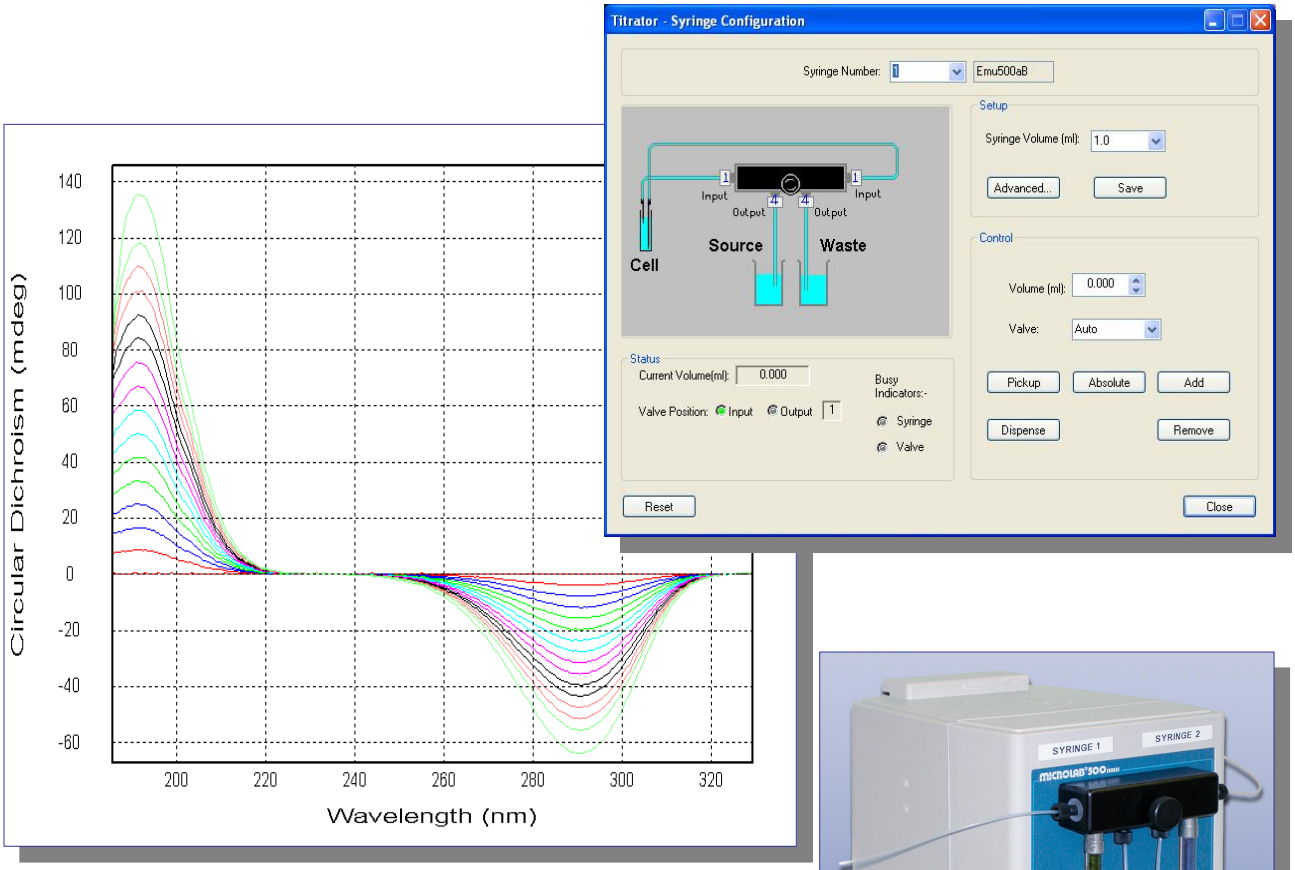
Accessory Features (PCM.3)

- **Compatible with all detection modes including fluorescence detection**
- **Temperature range -40°C to 105°C (with circulating chiller unit)**
- **Temperature slew rate 10°C per minute**
- **Magnetic stirring as standard in all four positions**
- **Optional cuvette temperature probes for monitoring sample temperature**
- **Total control via Chirascan software**
- **Extensive step-wise and continuous temperature ramping experimental options via Chirascan software**
- **Straightforward mounting in the sample handling unit**



TT.3 Dual-Syringe Titration Unit

The TT.3 accessory is a fully programmable, high accuracy titration system that enables automated measurement of concentration-dependent circular dichroism, fluorescence and absorbance data. Based on the Hamilton 500 series auto-dispenser unit, option TT.3 provides a 2-syringe reagent delivery system capable of accurately withdrawing and dispensing aliquots in a fixed volume cuvette to meet target concentrations pre-programmed by the user. All titration experiments are fully controlled via the Chirascan control software.



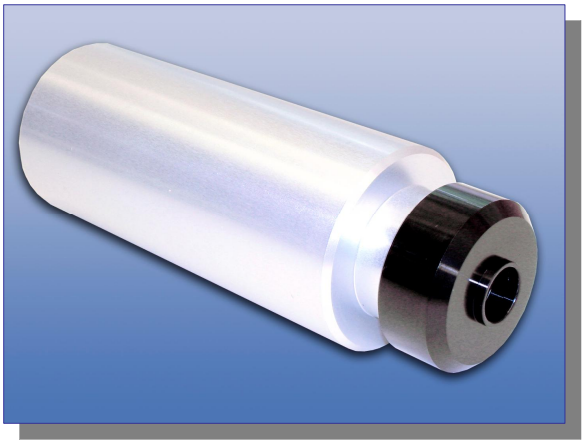
Accessory Features

- **Dual syringe concentration targeted mode**
- **Single syringe addition mode**
- **Full software control of single wavelength and scanning experiments**
- **Computer controlled stirrer capability supplied as standard**

TF.3 Total Fluorescence Detector

Chirascan may be equipped with an optional dedicated fluorescence detector for measurement of a total fluorescence or scattered light using the side port of the sample handling unit. The standard TF.3 detector comprises a housing fitted with a photomultiplier tube capable of measurement in the range 300-650nm (alternative PMTs are available for extended wavelength range operation) and detector mounting for the side port. It is suitable for measuring signal changes as a function of time, concentration or temperature and can also be used to record fluorescence excitation spectra.

Accessory Features



- **Standard option provides 300-650nm detection range**
- **Alternative detectors are available**
- **Distance from the detector to the sample cell is fully adjustable**
- **Detector contains a circular filter holder suitable for use with standard circular band-pass and interference filters (25mm diameter)**
- **May be operated using the standard CD channel or a dedicated fluorescence channel (option MC.3) which also provides simultaneous CD and fluorescence detection capability**

FP.3 Fluorescence Polarisation / Anisotropy Accessory

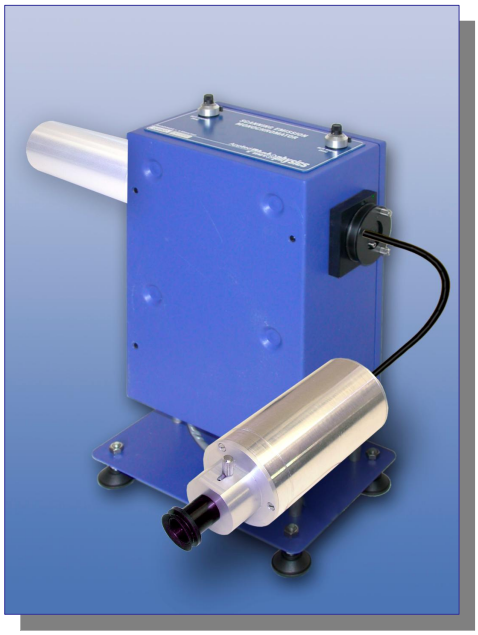
The FP.3 accessory employs Chirascan's photoelastic modulator (PEM) and a single photmultiplier detector for fluorescence polarisation/anisotropy measurements. This configuration provides greater sensitivity and a simpler instrument configuration than conventional fluorescence polarisation instrumentation.

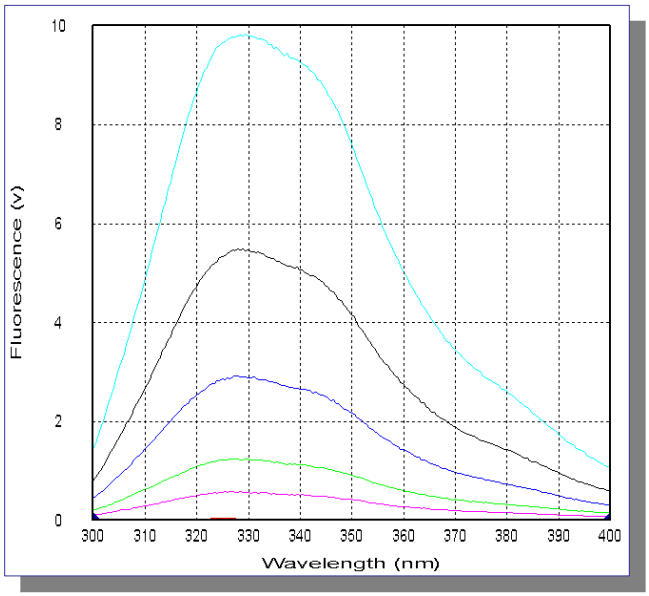
Conventional fluorescence polarisation measurements are made using a T-format design featuring two detectors and three polarisers; one for generating the polarised excitation light and two for collection of the horizontal and vertical components of the fluorescence signal from which the fluorescence polarisation (or anisotropy) value is derived. The sensitivity of this technique is limited by the use of the emission polarisers and differences in sensitivity between the fluorescence detectors.

Option FP.3 utilises Chirascan's PEM to generate vertical and horizontal polarised light eliminating the requirement for two detectors. Operating with a 100kHz modulation cycle, the single fluorescence detector detects both the horizontal and the vertical components of the emission. In addition, the absence of emission polarisers allows for the simultaneous collection of the total fluorescence signal. This approach also simplifies instrument configuration: the fluorescence polarisation detector mounts in side observation port of the sample chamber in the same way as for conventional total fluorescence measurements.

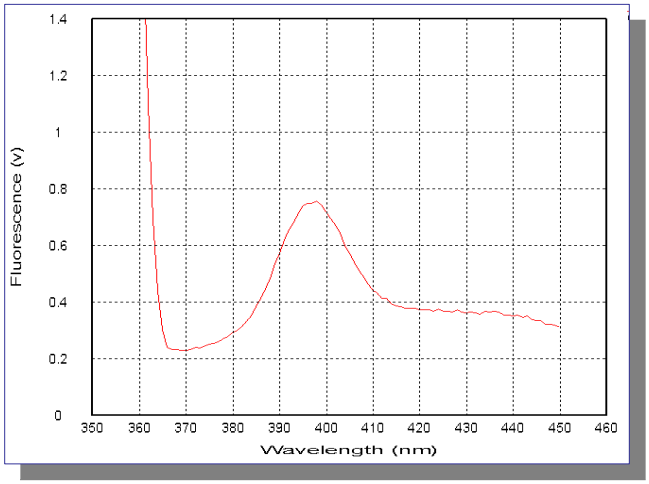
SEM.3 Scanning Emission Monochromator

The SEM.3 option is a software-controlled scanning emission monochromator and light guide which couples to the fluorescence port of the sample holder. With the TF.3 Total Fluorescence Detector, SEM.3 enables the collection of high quality fluorescence emission spectra.





Fluorescence emission spectra recorded using the SEM.3 and TF.3 accessories.



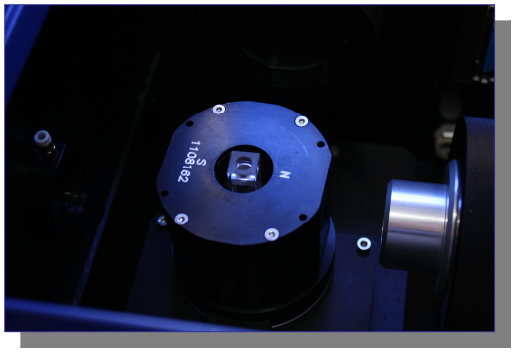
Fluorescence sensitivity is such that the Raman scattering of water is easily detectable. In the example (left), the unsmoothed CD data has a S/N ratio of over 70. If this data were smoothed (for example: to enable a direct comparison with data acquired on a spectrometer using electronic filtering) the S/N ratio would increase by a large factor.

Raman scattering of water recorded using the SEM.3 and TF.3 accessories.

Accessory Features

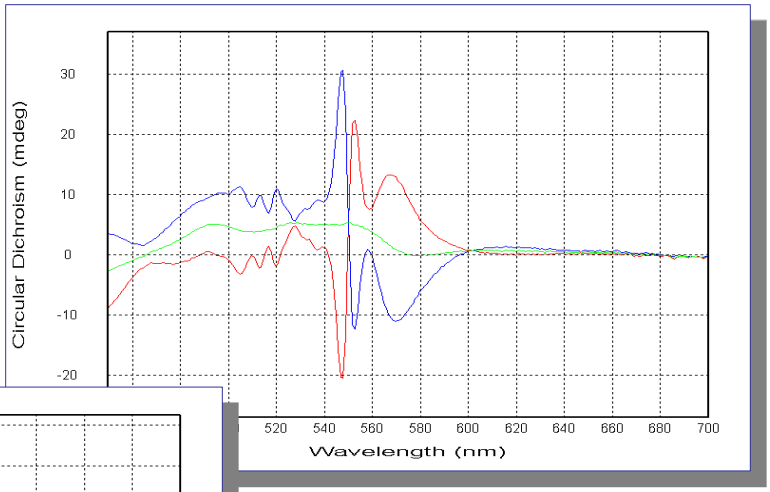
- **Single diffraction grating symmetrical Czerny-Turner optical layout**
- **Fibre-optic light-guide coupling between cuvette and monochromator entrance**
- **Full software control of scanning experiments**
- **May be operated using the standard CD channel or a dedicated fluorescence channel (option MC.3) which would also enable simultaneous CD and fluorescence signal detection capability**

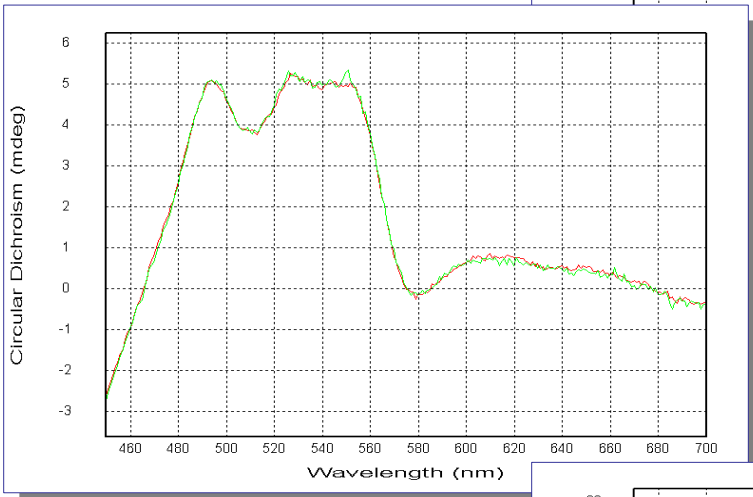
MCD.3 Magnetic Circular Dichroism



The MCD.3 accessory mounts directly into Chirascan's sample housing chamber and does not require further alignment or optimisation. The position of the CD detector can be easily moved along the optical axis and, for MCD measurements, it is set further back from the sample cell. Switching between CD and MCD detection modes is straightforward and takes less than 5 minutes. The field strength at the sample position is 1 Tesla.

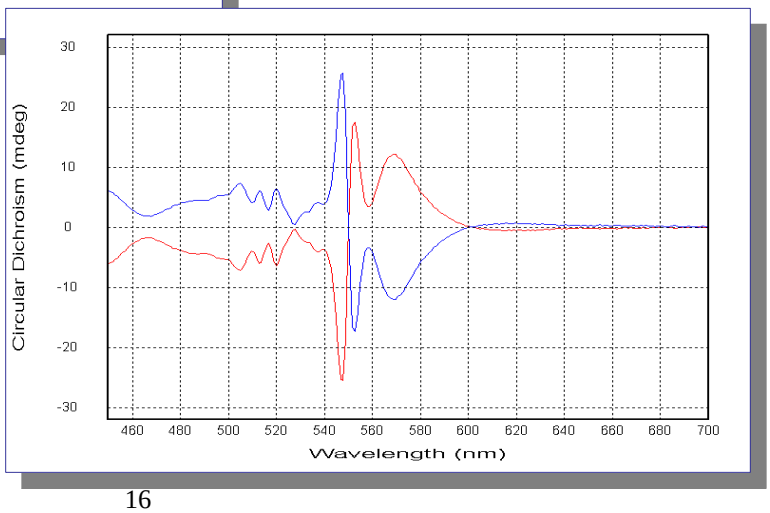
The spectra (right) show MCD spectra of Cyctrochrome C (blue and red traces). The scans are raw MCD data collected in 7 minutes - no smoothing (or filtering) has been applied. The average of these spectra (green trace) should, in theory, be the non-magnetized CD spectrum under these conditions.





The average MCD spectrum (green trace - left) is compared here with the measured CD spectrum of the same sample with the magnet removed (red trace). As can be seen, these spectra are virtually identical, underlining the accuracy of the MCD measurements.

The same MCD spectra are shown here after subtraction of the non-magnetized component from both. As would be expected from theory, these spectra are perfectly symmetrical about the X axis.

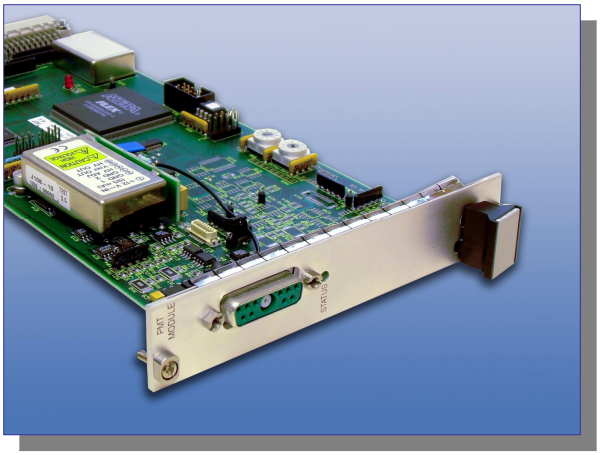


Fluorescence Detected Circular Dichroism (FDCD)

FDCD can be recorded using the standard Chirascan instrument after relocating the (CD) detector to the side observation port used for fluorescence measurements. If the user does not have the dedicated fluorescence detector option, it is recommended that a dedicated detector-mounting is purchased for this port to ease reconfiguration of the CD detector between the two ports.

MC.3 Multi-Channel Fluorescence

Chirascan may be equipped with an optional additional detection channel to provide the instrument with a dedicated fluorescence channel without the need to reconnect detector cables. Hence this option would usually be used in combination with the Total Fluorescence Detector, option TF.3 This multi-channel detection option will also allow simultaneous measurement of CD/absorbance and fluorescence signals. The accessory comprises an additional electronics module which is mounted in the Chirascan electronics rack and an additional detector cable.



Accessory Features

- **Fitted with 16bit A/D converter**
- **Used with the Total Fluorescence Detector (TF.3) provides a dedicated fluorescence detection channel without the need to reconfigure the instrument between CD and fluorescence modes**
- **Enables simultaneous CD/absorbance and fluorescence data acquisition**

J.3 Julabo AWC-100 recirculating cooler for use with the peltier

The peltier accessories PCS.3 and PCM.3 require water circulation for cooling. The J.3 is a compact water re-circulating cooler, specifically designed for removing small heat loads from external systems such as Peltier-elements.

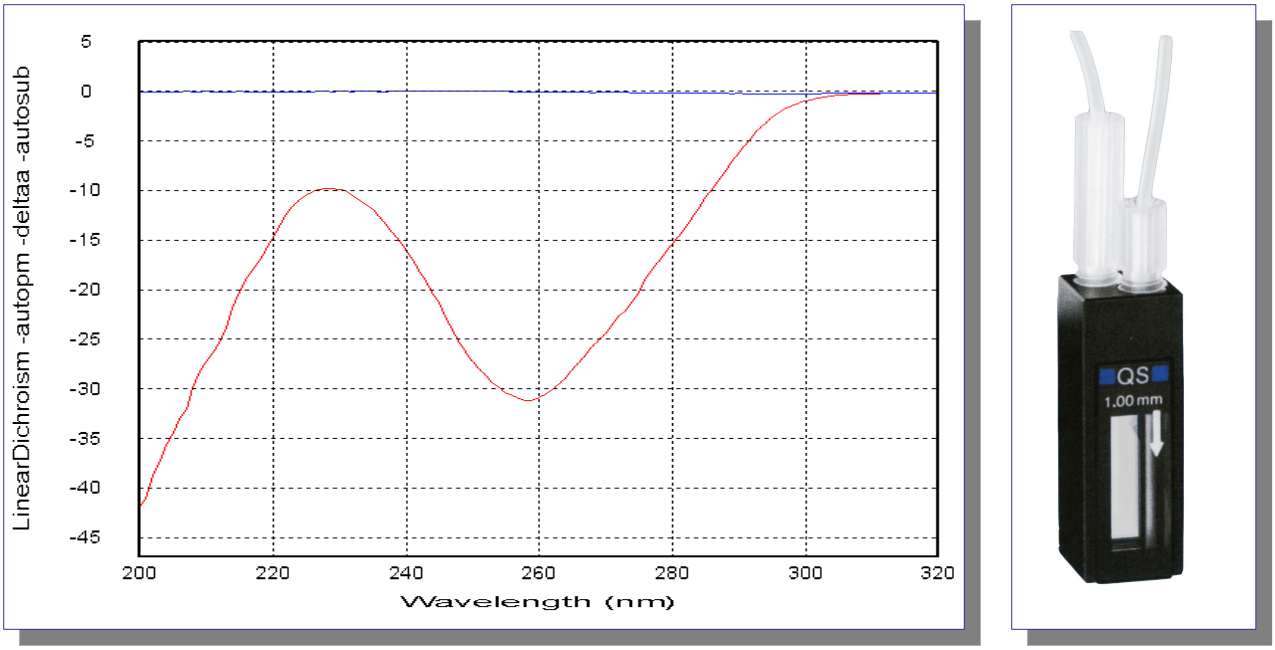
- **Small foot print**
- **Virtually noiseless**
- **Easy to use**
- **Economical**

Filling Volume: 0.85 Liters
Dimensions (W x L x H): 20.5 x 36 x 31.5 cm
Weight: 11 kg

LD.3 Linear Dichroism Detector

Linear dichroism is the difference in the absorption of parallel and perpendicular linearly polarised light. Chirascan can be enabled for measurement of LD spectra simply by switching from the standard detector to the LD.3 detector; this comprises a photomultiplier detector and amplifier tuned to twice the PEM modulation frequency.

Sample handling and orientation is left to the discretion of the user. Most commonly a flow through cell is used, such as the one pictured, and sample is continuously pumped through the beam path to promote sample orientation. The use of a *Couette* flow cell is also well established[1,2].



The LD spectrum (above) is of Calf Thymus DNA under the conditions tabulated below. This is raw data; no post-acquisition smoothing (or filtering) has been applied. Also shown (blue trace) is the LD spectrum obtained from the same sample when there is no flow (i.e. no sample orientation).

Sample	**Calf Thymus DNA**
Solvent	**10mM pH 7 sodium cacodylate buffer**
Concentration	**5mg/ml (approx)**
Step size	**1.0nm**
Bandwidth	**1nm**
Time per point	**1s**
Pathlength	**0.5mm**
Duration of measurement	**4 minutes**

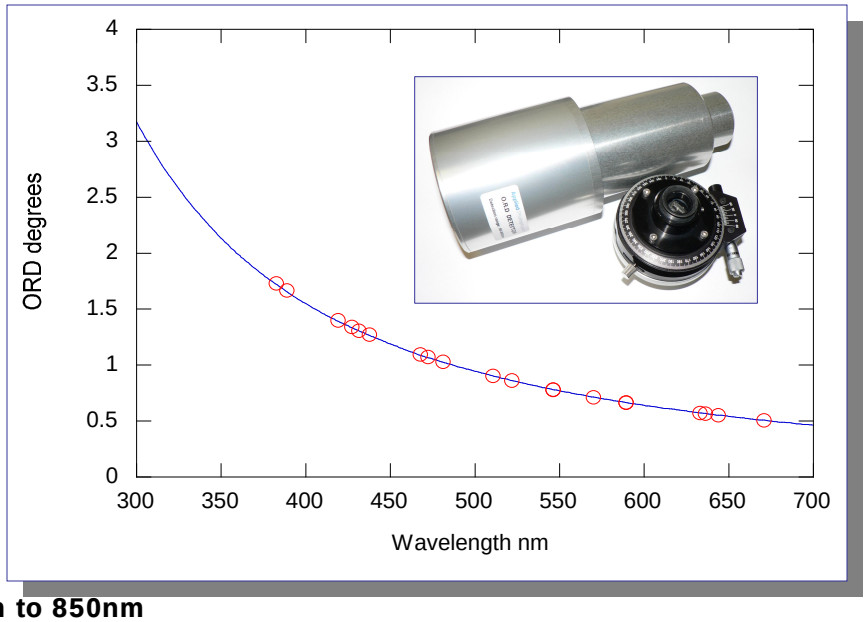
[1] Wada, A., and S. Kozawa. *1964. Instrument for the studies of differential flow dichroism of polymer solutions. Journal of Polymer Science Part A. 2:853–864.*

[2] Rachel Marrington, Timothy R.Dafforn, David J. Halsall and Alison Rodger, *Biophysical Journal 87:2002-2012 (2004) Micro-Volume Couette Flow Sample Orientation for Absorbance and Fluorescence Linear Dichroism*

ORD.3 Optical Rotary Dispersion Accessory

Optical Rotary Dispersion is a measure of the rotation in the plane of linearly polarised by the sample. In effect, it is a measure of the difference in refractive indices of the sample for left and right circularly polarised light. Chirascan's ORD.3 accessory enables measurements of ORD spectra and is based on the method of Shindo *et al*[1]. The accessory consists of a linearly polarising analyser prism on a rotatable mount and a dedicated detector. The photoelastic modulator (PEM) of the Chirascan is automatically configured by software as a dynamic half-waveplate to output alternating vertical and horizontally polarised light. The analyser prism is placed after the sample and before the detector at a 45° angle to the alternating vertical and horizontal light (the null point). The difference in signal is obtained using a dedicated high gain photomultiplier detector tuned to twice the PEM modulation frequency.

The ORD.3 accessory doesn't use the optical nulling method used by most commercial polarimeters and so no physical rotation of an analysing polariser is required during data acquisition. The advantage of this approach is the ability to rapidly scan an ORD spectrum. The figure below shows a comparison of an ORD spectrum of a solution of sucrose (100mg/ml) compared to optical rotations from literature values at a number of single wavelengths[2,3]. This ORD spectrum was collected from 300 to 700 nm with a 1nm data pitch in approximately 6.5 minutes. Higher scanning speeds are easily achievable.



ORD spectrum of 100mg/mL solution of sucrose in water (10mm pathlength cell) recorded on Chirascan with the ORD.3 accessory (blue line). Literature values at a number of selected wavelengths[2,3] are overlaid (red circles).

Accessory features

- **Wavelength range: 215nm to 850nm**
- **Detection range: -5° to +5°**
- **Detection limit: 0.1 mdeg**

[1] Y. Shindo, H. Hayakawa, and M. Sudani, *Appl. Spectrosc.* 1989, 43, pp. 1471-1475.

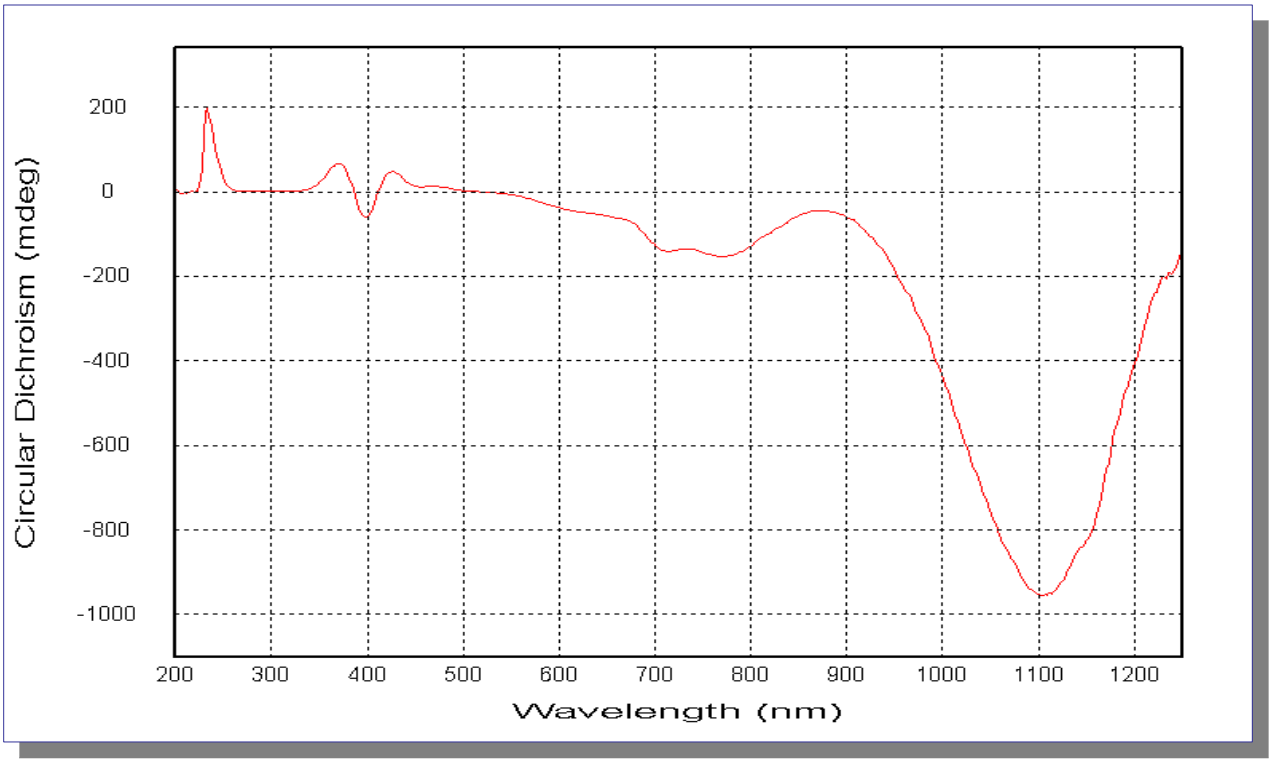
[2] National Physics Laboratory, Kaye and Laby Table of Physical and Chemical Constants. Web Edition. 2007, www.kayelaby.npl.co.uk. Section 2.5.10.

[3] T.M. Lowry, *Optical Rotary Power*, 1935, Longmans, Green and Co

IR.3 Extended NIR Photomultiplier Detector

The limit of detection in the NIR wavelength region can be extended from 900nm, with the standard detector, to 1250nm with the IR.3 detector.

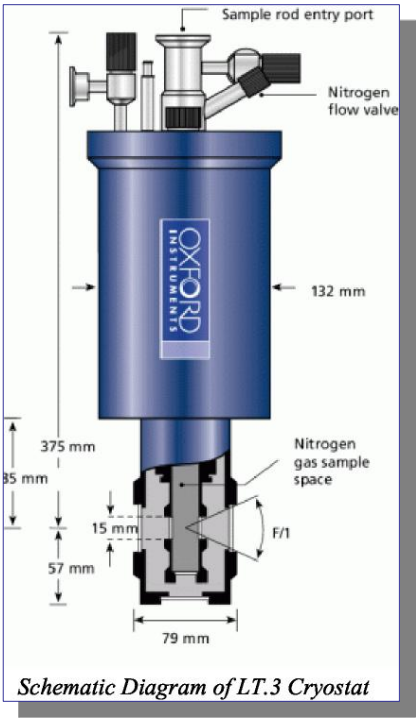
Nickel tartrate is a CD standard that is used to demonstrate performance from the near-UV into the near-infrared. The figure (right) shows the CD spectrum of nickel tartrate collected using IR.3 detector under the experimental conditions tabulated. The CD spectrum shown here is the raw (unsmoothed) spectrum.



Sample	**Nickel Tartrate**
Concentration	**0.24M**
Step size	**2.0nm**
Bandwidth	**10.0nm**
Time per point	**0.5s**
Pathlength	**10mm**
Duration of measurement	**< 5 minutes.**

This IR.3 detector is suitable for CD measurements over the range < 250nm to 1250nm.

LT.3 Nitrogen Cryostat Accessory



Schematic Diagram of LT.3 Cryostat

Chirascan may be equipped with a nitrogen cryostat for low temperature CD measurements. The cryostat interfaces neatly with the Chirascan sample housing. The optimised thermal design provides excellent control and stability of the sample temperature.

The LT.3 cryostat is a top loading, static exchange gas cryostat. The sample is located in a central space surrounded by an exchange gas (typically nitrogen) providing extremely uniform cooling.

Changing the sample simply involves removing the sample rod maintaining overpressure of exchange gas, replacing the sample and inserting the rod back into the cryostat. There is no need to break the insulating vacuum and warm the cryostat up. The resulting sample change times are very short, typically a few minutes.

Accessory Features

- **77K to 300K temperature range**
- **Temperature stability of ± 0.1K (10 minute period)**
- **20min cool down time (ambient to 77K)**
- **Liquid nitrogen capacity 1.2L**
- **15 hour cryogen hold period before refill is necessary**
- **5 minute sample change time**
- **Sample holder dimensions: Width 19mm and height 30mm. Optical sample holder features a 15mm aperture**

CFR.3 21 CFR Part 11 Compliance

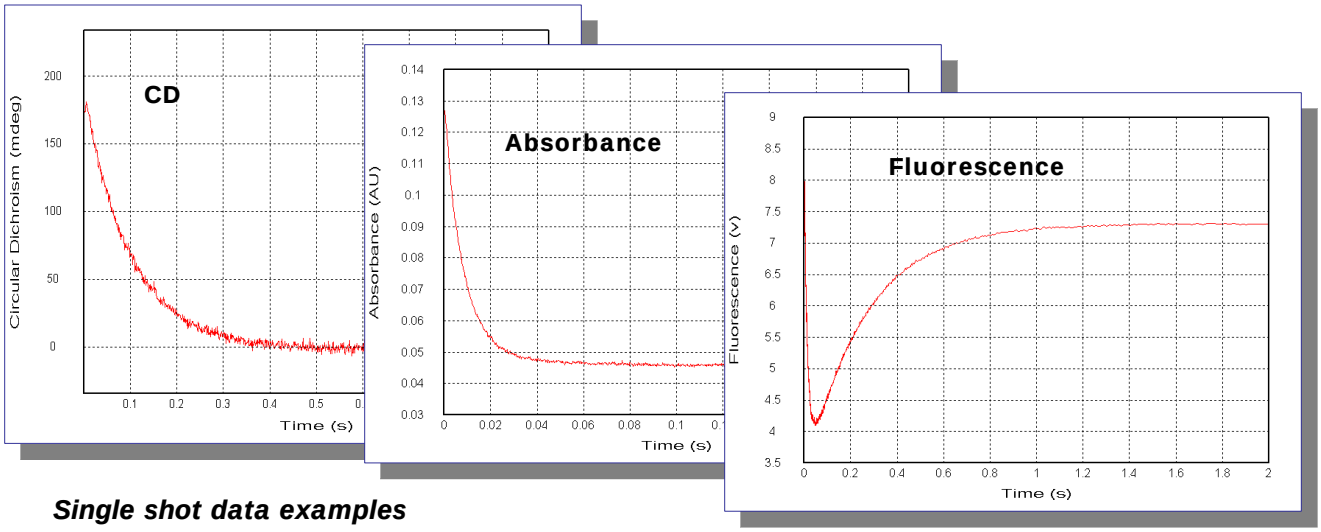
Applied Photophysics Ltd (APL) can supply Chirascan CD spectrometer systems which are installed to be 21 CFR Part 11 (CFR) compliant. CFR compliance is achieved through a combination of enforced administrative procedures, secure software, and secure data structures.

Our implementation 21 CFR Part 11 ensures that only authorized personnel can perform experiments, collect data, and subsequently perform any data manipulations. Furthermore, the software will ensure that all modifications are logged. Users which should not be able to make modifications to data (including network administrators etc.) cannot modify data without leaving evidence of that modification.

SF.3 Stopped-Flow Unit

The SF.3 accessory brings to Chirascan and Chirascan-plus our world-leading expertise in stopped-flow design to provide rapid kinetic CD, absorbance and fluorescence measurements of unsurpassed sensitivity. The unit is based on our best-selling SX20 and Pi-Star stopped-flows, and designed to enable rapid and straightforward coupling: a roller-plate mount enables it to be easily located and locked into place at the exit slit of the Chirascan monochromator (in place of the sample chamber unit).

A demountable stopped-flow cell provides optical pathlengths of 2mm and 10mm and has a dead-time of 1.5ms. Cells are rapidly interchangeable and a shorter dead-time cell is available as an option. CD and absorbance kinetics are collected using the standard Chirascan detector, and fluorescence kinetics with the TF.3 detector option.



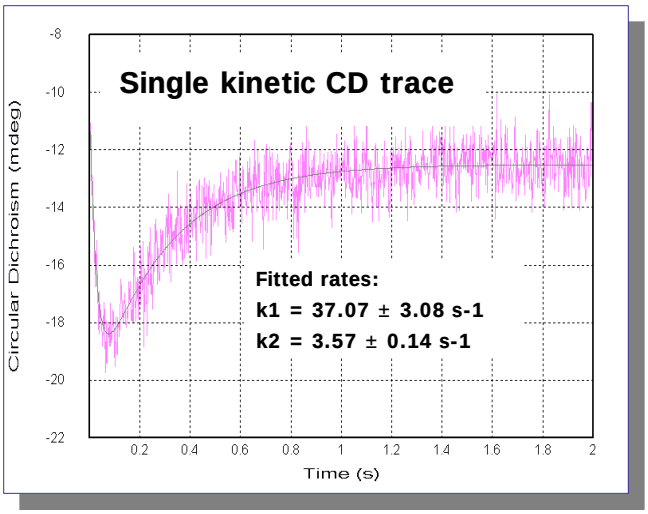
Single shot data examples

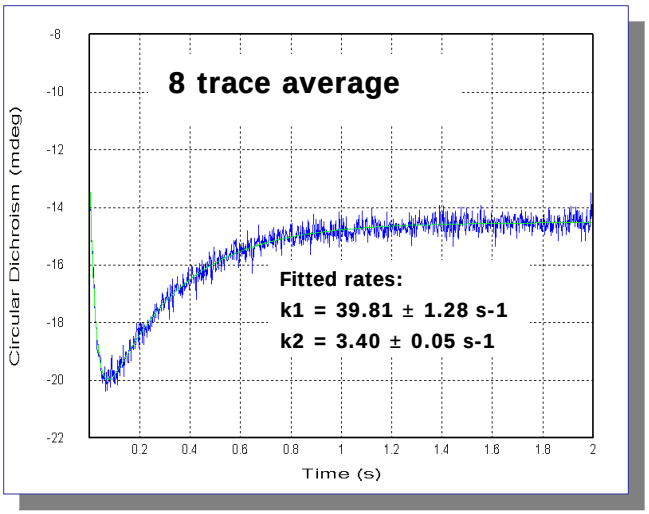
Accessory Features

- **Optimised for absorbance and fluorescence detection without the need for reconfiguration.**
- **Straightforward bench-top configuration. Can be fitted in 10 minutes.**
- **Low sample volume requirement**
- **Flow circuit materials suitable for anaerobic experiments and aggressive reagents**
- **Short optical pathlength for fluorescence detection (minimises inner-filtering effect).**
- **Large ratio-mixing capability; up to 25:1**
- **Comprehensive range of accessories available including sequential-mixing (SQ.3), fluorescence polarisation (FP.3) and dual fluorescence detection (DF.3)**

The example below, recorded on Chirascan with the SF.3 accessory, shows the refolding of lysozyme from 6M GuHCl by a 1 in 10 dilution in buffer. Below left is a single stopped-flow drive monitored at 225nm using a Xe-Hg. Below right is an 8 shot average.

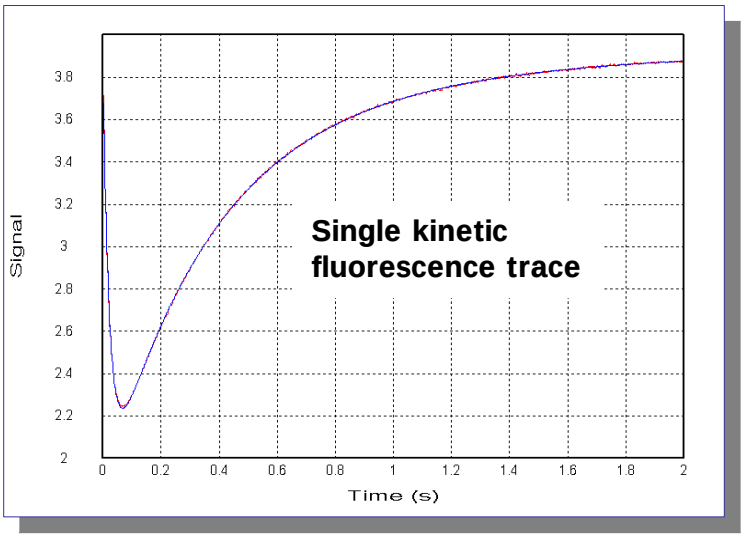
The total volume requirement (lysozyme and buffer) is 250ul per drive or 22.5ul of lysozyme per drive (= 50ug).





We are confident that competitor CD-stopped-flows would have to average well over 25 traces to obtain equivalent data quality to the single kinetic trace shown above.

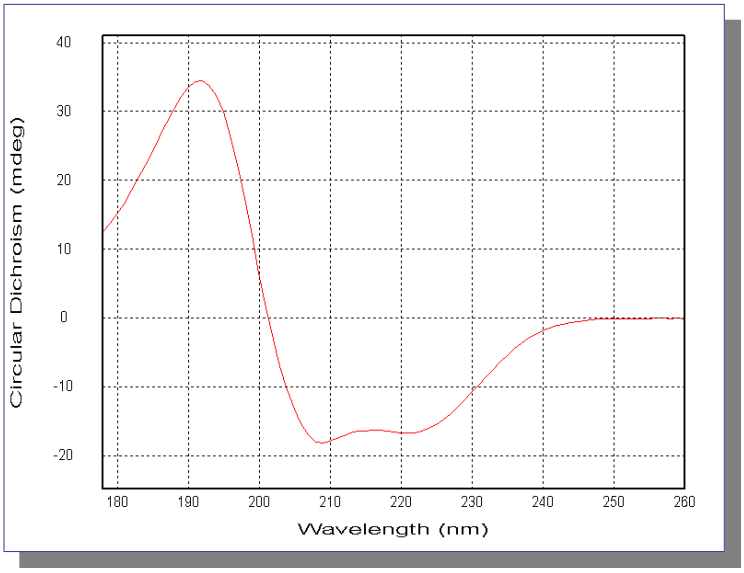
Note: with the Chirascan-*plus* spectrometer, the sensitivity is even greater than shown here (3 to 4 fold improvement)

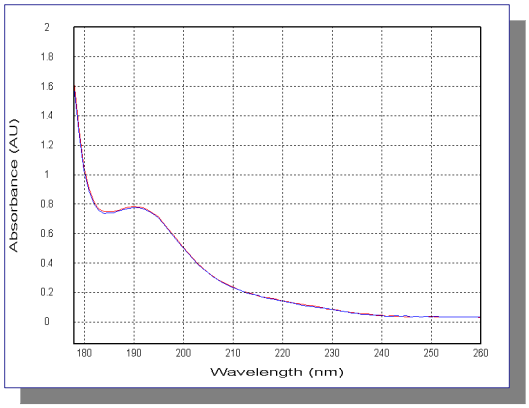


The stopped-flow fluorescence trace shown above (using the TF.3 detector option) shows the refolding of lysozyme from 6M GuHCl by a 1 in 10 dilution in buffer. This is a single trace with excitation at 285nm and using a 305nm Cut-off filter. The fitted trace (blue) is overlaid.

Data Examples (recorded on a standard Chirascan)

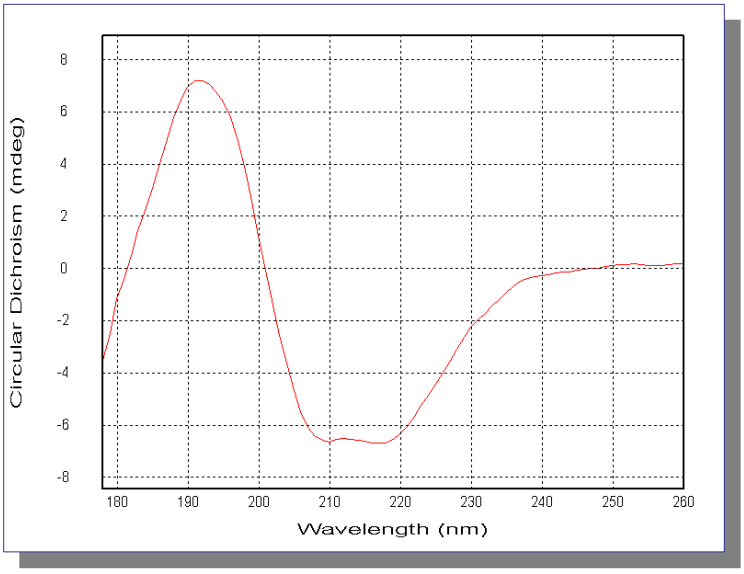
Bovine Serum Albumin

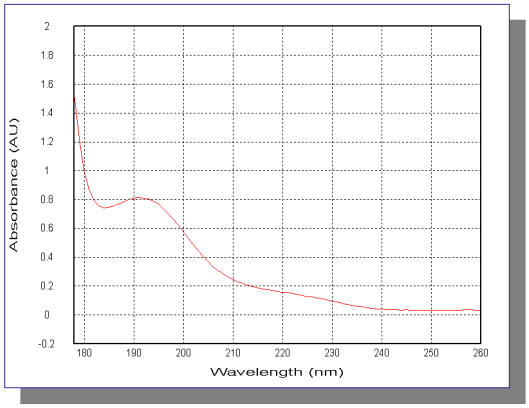




Sample	BSA
Concentration	1mg/mL
Wavelength Range	178 – 260nm
Step size	1.0nm
Bandwidth	1.0nm
Pathlength	0.1mm
Duration of measurement	30 seconds (164nm/min)
Post-acquisition smoothing	4 point smooth

Alcohol Dehydrogenase

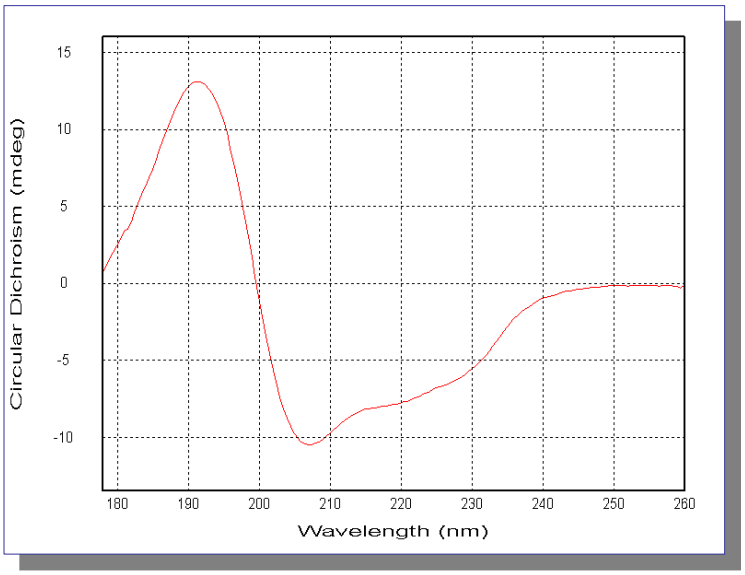


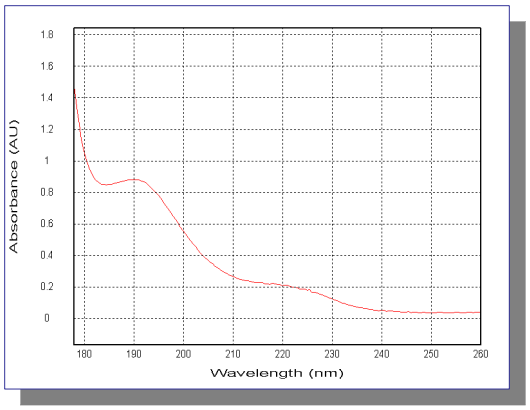


Sample	Alcohol Dehydrogenase
Concentration	1mg/mL
Wavelength Range	178 – 260nm
Step size	1.0nm
Bandwidth	1.0nm
Pathlength	0.1mm
Duration of measurement	30 seconds (164nm/min)
Post-acquisition smoothing	4 point smooth

The inserts on the right show the corresponding absorbance spectra collected simultaneously with the CD data.

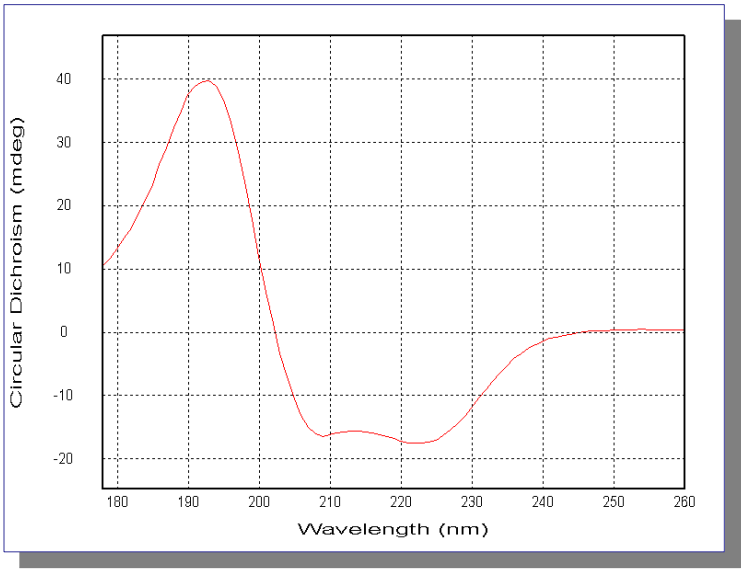
Lysozyme

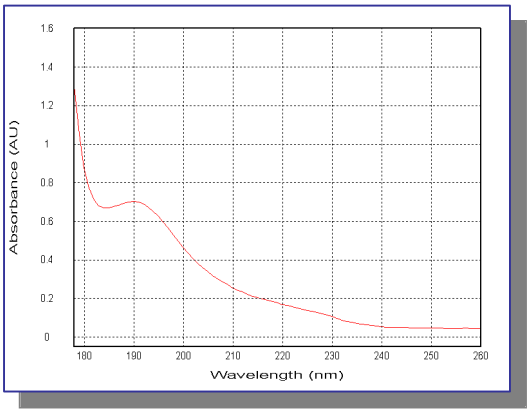




Sample	Lysozyme
Concentration	1mg/mL
Wavelength Range	178 – 260nm
Step size	0.5nm
Bandwidth	1.0nm
Pathlength	0.1mm
Duration of measurement	30 seconds (164nm/min)
Post-acquisition smoothing	6 point smooth

Myoglobin

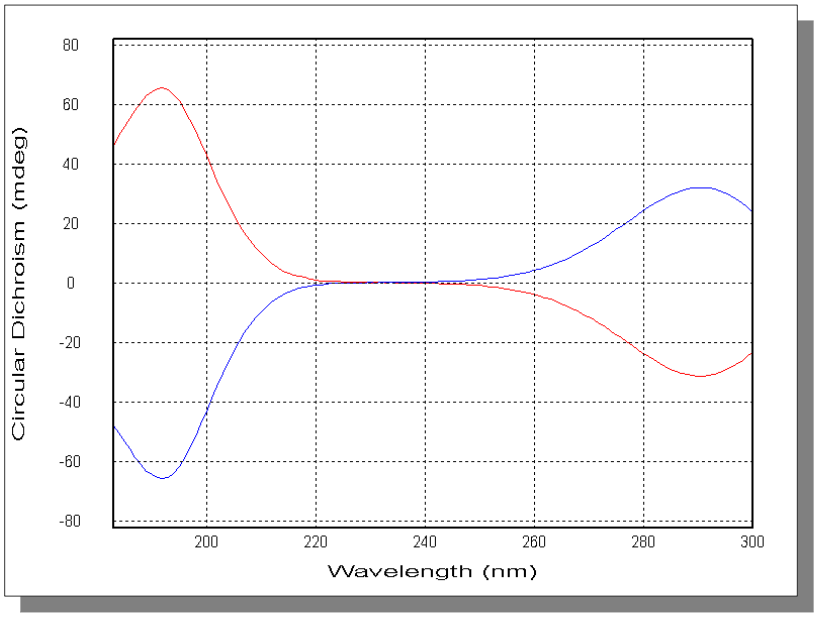


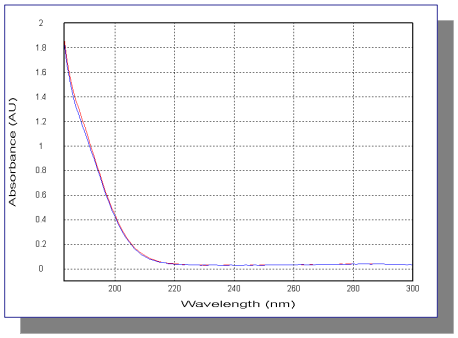


Sample	Myoglobin
Concentration	1mg/mL
Wavelength Range	178 – 260nm
Step size	1nm
Bandwidth	1.0nm
Pathlength	0.1mm
Duration of measurement	30 seconds (164nm/min)
Post-acquisition smoothing	RAW data (no smoothing)

The inserts on the right show the corresponding absorbance spectra collected simultaneously with the CD data.

Camphorsulfonic Acid (CSA) and Ammonium Camphorsulfonate (ACS)

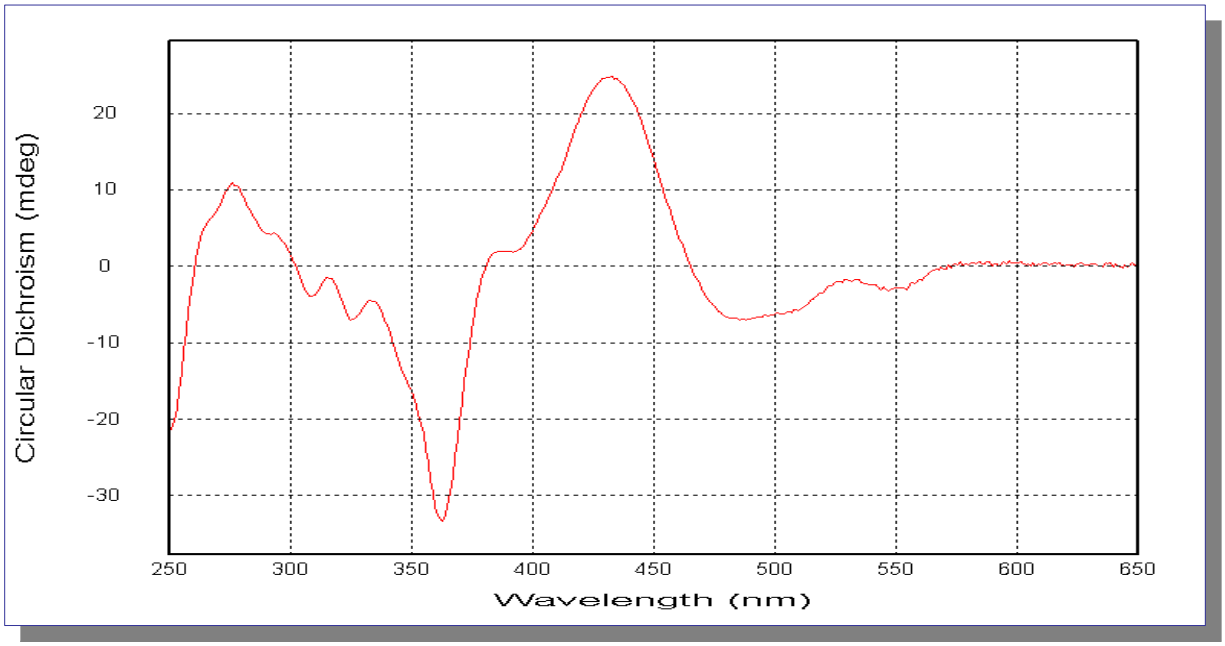




Sample	ACS (red trace) CSA (blue trace)
Concentration	1mg/mL
Wavelength Range	183 – 260nm
Step size	1nm
Bandwidth	1.0nm
Pathlength	1mm
Duration of measurement	3 minutes each (26nm/min)
Post-acquisition smoothing	RAW data (no smoothing)

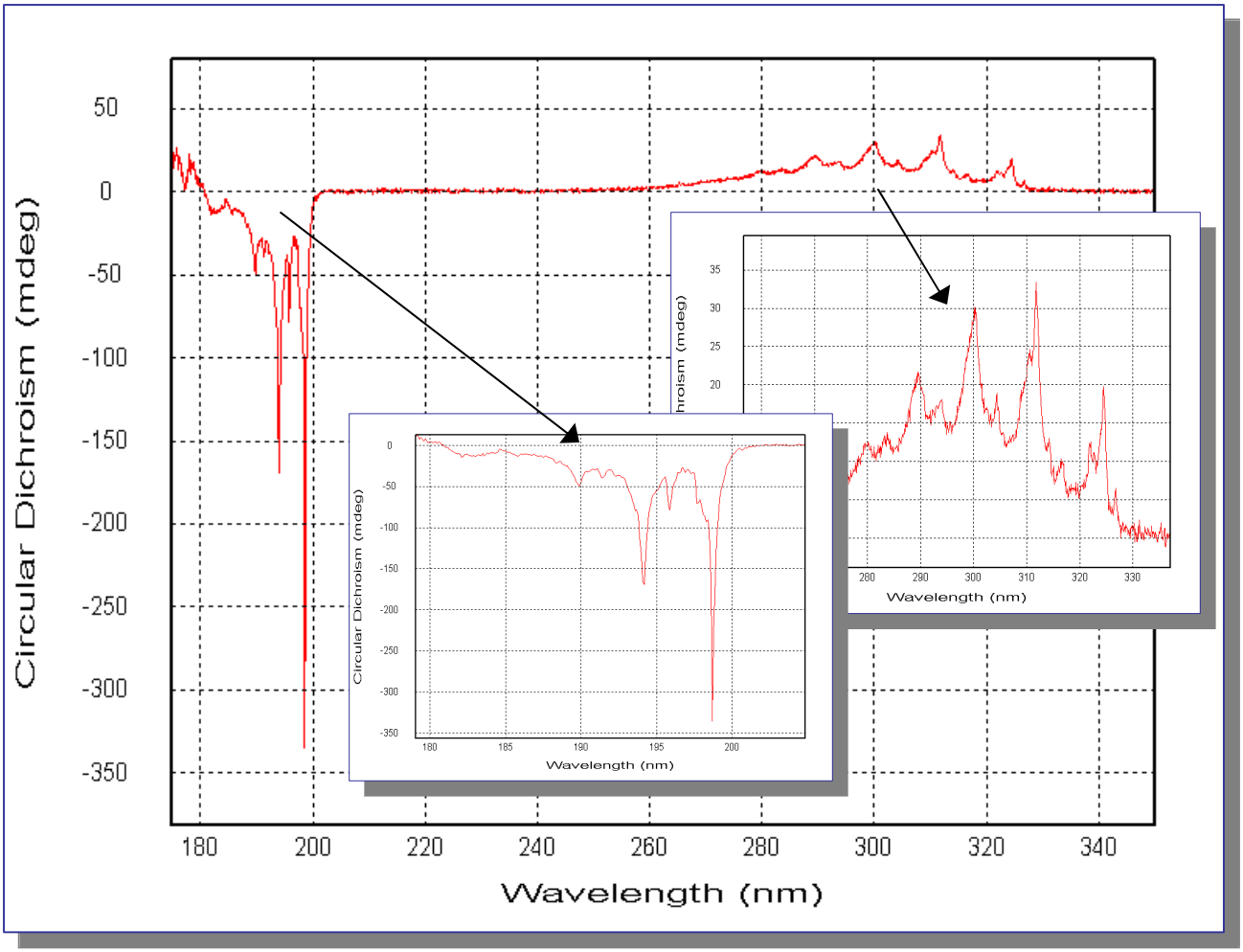
The insert shows the corresponding absorbance spectra acquired with these CD spectra.

Vitamin B12



Sample / concentration	Vitamin B12 / 0.2mg/mL
Wavelength range	800nm – 250nm
Bandwidth	1nm
Step size	1nm
Duration of each scan	~7 minutes (~80nm/min)
Pathlength	5mm
Post-acquisition smoothing	RAW data (no smoothing)

(R+)3-Methylcyclopentanone



Sample	(R+)3-Methylcyclopentanone
Concentration	Vapour
Wavelength Range	175 – 350nm
Step size	0.1nm
Bandwidth	0.1nm
Pathlength	10mm
Duration of measurement	32 minutes (5.5nm/min)
Post-acquisition smoothing	RAW data (no smoothing)

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