AppliedPhotophysics

Chirascan™-plus DMS

for the most demanding applications in CD spectroscopy

Structural and thermo-dynamic data in one hour with as little as 50 μg of protein

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Chirascan-plus DMS

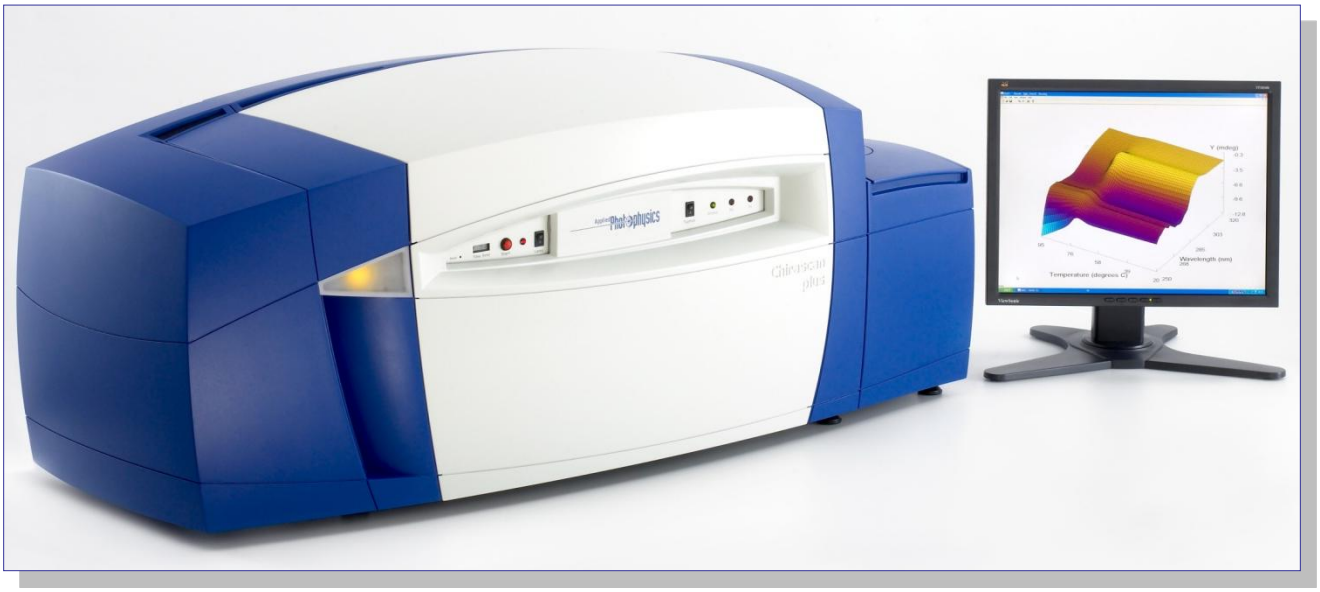
CONTENTS

Introduction

Chirascan-plus and **dynamic multi-mode spectroscopy (DMS)** were developed to measure complete far-UV and near-UV spectra during thermal denaturation to yield structural and thermodynamic information on proteins. These measurements are of great importance to the biopharmaceutical industry in identifying stable, well-formulated proteins of consistent quality and structural integrity and Applied Photophysics has worked closely with a number of pharmaceutical companies to develop **Chirascan-plus DMS**.

The effectiveness of the **DMS** technique is based on its high data content, fast data acquisition and low sample volume requirement. It is the result of a significant advance in the speed and sensitivity with which CD spectra can be collected using the new **Chirascan-plus** spectrometer. Chirascan-plus delivers the performance needed to complete a full-spectrum, continuous-ramp thermal denaturation experiment in about one hour and using only a small volume of sample. **Global 3** global analysis software has been developed to take full advantage of the richness of the acquired data to generate spectroscopic and concentration profiles of all the contributing species, and calculate the mid-point temperatures and van't Hoff enthalpies of the phase transitions. **Chirascan-plus DMS** is a fully optimised solution with wide application in the field of drug discovery and development - for example in:

- Lead optimisation
- Protein engineering
- Small & large drug binding
- Membrane studies
- Formulation and stability testing
- Biocomparability & quality control
- Sructure activity relationship (SAR) studies

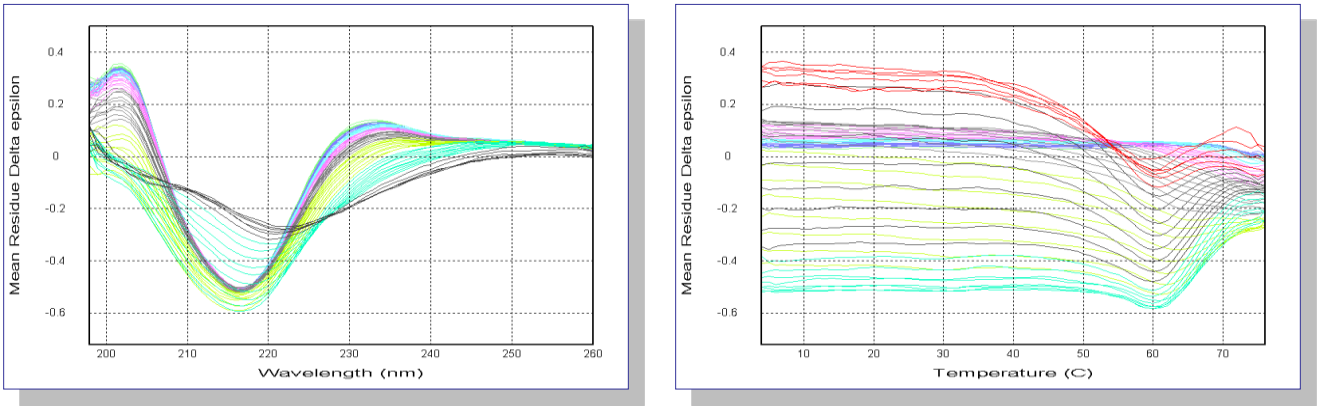


Dynamic multi-mode spectroscopy

Dynamic multi-mode spectroscopy (DMS) is the name given to a new experimental technique developed at Applied Photophysics for determining how a protein or other bio-macromolecule (e.g. DNA, RNA) behaves when subjected to changing, usually increasing, temperature. The stability of a protein is conventionally monitored by differential scanning calorimetry (DSC) to yield thermodynamic parameters such as the enthalpy and the mid-point of a conformational transition but each phase transition must be accompanied by a distinct change in heat capacity in order to be clearly identified. In contrast, **DMS** uses circular dichroism (CD) to monitor protein conformation directly and hence all phase changes (conformational changes) can be more readily identified.

CD is the only common method for determining and monitoring protein secondary structure in solution and so, unlike calorimetric techniques, **DMS** also provides structural information. Knowledge of secondary structure will confirm whether or not the protein is correctly folded and hence biologically active, monitoring secondary structure as a function of temperature will identify what structural components change and the order in which they denature, and measurement of the corresponding absorbance and/or fluorescence spectra enables unfolding and aggregation steps to be distinguished, of potential relevance to future immunogenicity.

Conventionally, measuring CD as a function of temperature can be time-consuming; denaturation experiments are carried out either as a continuous temperature ramp at a single wavelength or as a series of spectra measured after thermal equilibration at discrete temperatures. Neither technique is ideal; in the former the behaviour of the protein at a single wavelength is assumed to be representative of all wavelengths. In the latter case, the time taken to perform the experiment (several hours) is prohibitive and kinetic effects are likely to influence the result. **DMS** combines these two methods into a single experiment which is quick and easy to perform, thereby eliminating kinetic effects (the total acquisition time being equivalent to a DSC experiment) and generating data across the whole wavelength range of interest. In addition, global analysis of the entire data set to the transition model (i.e. the denaturation curves at *all* wavelengths) leads to a more robust determination of the thermodynamic parameters.



A **DMS** experiment always records the CD and absorption spectra simultaneously and, if required, the fluorescence excitation spectrum can also be recorded. In a typical **DMS**

DMS circular dichroism data

experiment in the far-UV, approximately 70μg of sample is required, spectra are recorded at temperature intervals of about 1^0C, and each spectrum is recorded at discrete wavelength steps (typically 1nm). The duration of the experiment is determined by the rate of the temperature ramp (usually 1^0C per minute) the temperature range. For the **DMS** data shown above the temperature range was 4^0C to 76^0C and so the duration of the experiment was 72 minutes.

Global 3 software is used to globally analyse the data; calculating the phase transition mid-point temperatures and the van't Hoff enthalpies of transition, and determining the spectroscopic and concentration profiles of all the contributing species. The changes in the CD spectra accompanying each phase transition can of course be viewed directly and aggregation, if present, can be identified from inspection of the absorbance spectra.

Development Background

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/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.

2004 saw the release of **Chirascan**; the result of over 3 years research and development, Chirascan has set new standards for steady-state circular dichroism spectroscopy; incorporating innovative optical design features to maximise light throughput (particularly in the far-UV wavelength region) and achieving light levels far beyond that of any other CD spectrometer. Unlike conventional CD spectrometers which use analogue electronic filters to irreversibly smooth (and potentially distort) CD spectra during acquisition, Chirascan uses a sophisticated digital data acquisition system that facilitates the rapid collection of more accurate CD spectra. Data smoothing, if required, is post-acquisition and fully reversible. This approach also simplifies operation; Chirascan is as straightforward to use as a single-beam spectrophotometer.

Chirascan-plus is an enhancement of the standard Chirascan spectrometer which offers truly remarkable speed, sensitivity and flexibility. Chirascan-plus builds on Chirascan's high light throughput with the introduction of a new, very high performance, solid state detector coupled with advanced intelligent electronics. The result is improved sensitivity and speed; in the far-UV wavelength region, equivalent quality CD spectra can be collected 4 times faster than with Chirascan. Other benefits include; wider wavelength range, more accurate absorbance measurement (simultaneous with the CD measurement) and flatter baselines.

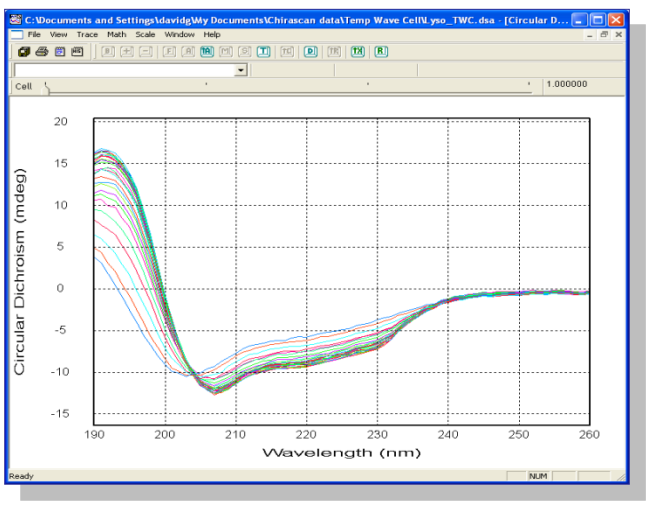
Whilst the signal-to-noise advantage of **Chirascan-plus** will benefit very demanding applications and techniques such as CD stopped-flow, it also offers a whole new approach to experimental design based on high data content, fast data acquisition and low sample volumes. These features have wide application in the pharmaceutical sector where improved productivity is so important and **Chirascan-plus DMS** is designed principally for these applications.

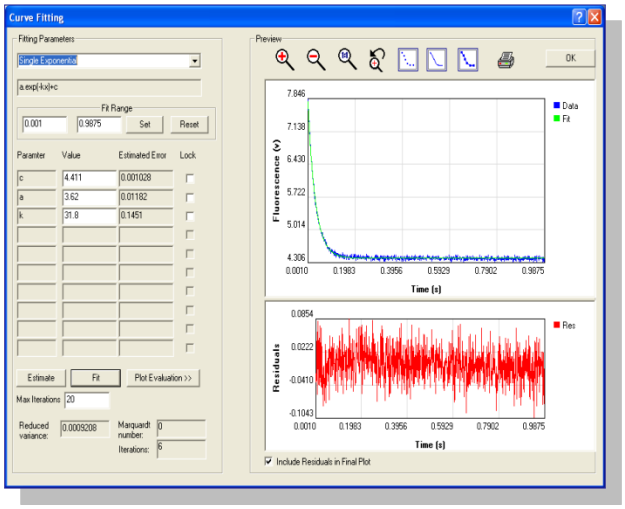
Instrument Features

This section is divided into 3 parts; standard features of all Chirascan spectrometers, additional features associated with the Chirascan-plus spectrometer, and additional features of the Chirascan-plus DMS package.

I - Chirascan spectrometers: standard features

- Exceptionally high sensitivity and light throughput. Innovative optics provide vastly superior light throughput compared to older designs, resulting in faster, low-noise data acquisition
- Digital acquisition / no electronic filtering. Guarantees data fidelity and avoids the risk of distortion associated with the use of electronic time constants
- 5 detection channels: CD, Absorbance/Transmission, HT, Temperature and Voltage. Simultaneous multi-channel data acquisition ensures all key information is recorded with every measurement
- Pro-Data control software running on Windows™ with comprehensive acquisition, display and analysis tools including: live display, advanced monochromator settings, kinetic acquisition and analysis, post-acquisition smoothing, straightforward conversion to other file formats. Unlimited licence to install Pro-Data on desktop computers is included.



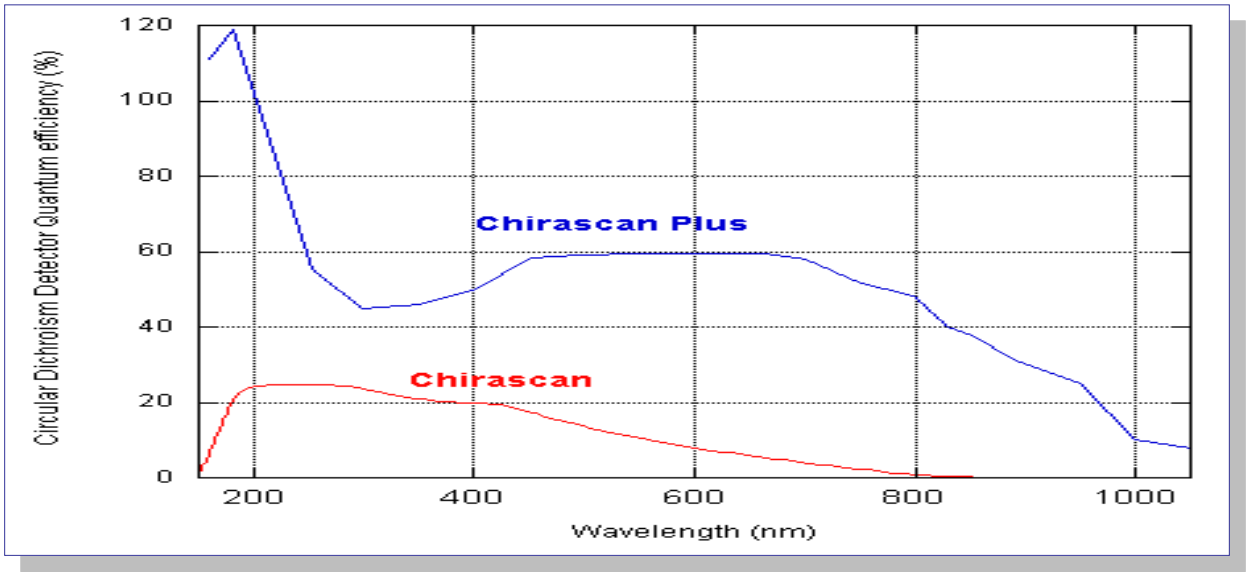


- Very low nitrogen consumption keeps running costs low. Rapid and efficient nitrogen purging ensures that just 5 l/min^{-1} is required for far-UV work, even on start-up and after **Chirascan** has not been in use for several days
- Moveable detector. The distance between the cell and the detector is easily adjustable ensuring that performance can be optimized for 'difficult' reagents, e.g. turbid solutions
- CDNN secondary structure analysis software
- 24 month warranty

II - Chirascan-plus spectrometer: features

Chirascan-plus (part no. **CS/3**) 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 with the introduction of a new, very high performance, solid state avalanche photodiode detector coupled with advanced intelligent electronics. **Chirascan-plus** offers:

- Improved sensitivity. The high quantum efficiency of the solid state detector in comparison with the standard photomultiplier detector (see figure below) leads directly to improved signal-to-noise over the standard Chirascan spectrometer. 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 to Chirascan can be achieved with a 4-fold increase in scanning speed



- Wider wavelength range: 163–1100nm (c.f. 163-900nm with the standard Chirascan). The signal-to-noise advantage increases significantly at visible wavelengths and into the NIR
- Flatter baselines. A significant contributor to baseline offsets in CD spectrophotometers is caused by the photomultiplier window itself. The solid-state detector does not have a window and so contributes much less to the baseline.
- 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.
- 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

III - Chirascan-plus DMS: features

Chirascan-plus DMS comprises part no. **CS/3d** (Chirascan-plus spectrometer with PC) and accessories; **CS/PCS** (single cell peltier temperature controller) and **CS/DMS** (see below).

Chirascan-plus DMS is the optimized solution for biotherapeutic drug development, formulation testing and other pharmaceutical applications. Fully integrated software and optimised components, coupled with the superior performance of Chirascan-plus, make this the ideal tool for demanding applications where top performance is required in terms of sample throughput, acquisition time and data quality.

CS/DMS comprises the following components:

- **CS/TF Total Fluorescence detector** and **CS/MC Multi Channel detection**
- Rectangular cells for far-UV measurements: 4 off 0.5mm (90µl volume)
- Rectangular cells for near-UV measurements: 4 off 10 x 4mm (500µl volume)
- Customised cell carriages for far-UV and near-UV cells
- Customised thermocouples: 2 off 0.25mm diameter, 1 off 1mm diameter
- **GLOB3 Global 3 global analysis software**
- **CS/CFR 21 CFR Part 11 Compliance software**

- The **CS/TF Total Fluorescence detector** and **CS/MC Multi Channel detection** enable simultaneous detection of fluorescence excitation spectra with the CD/absorbance measurement using the side port of the sample handling unit. The standard **CS/TF** detector is a photomultiplier tube capable of measurement in the range 300-650nm (alternative PMTs are available). **CS/TF** is also suitable for measuring fluorescence changes as a function of time, concentration or temperature.

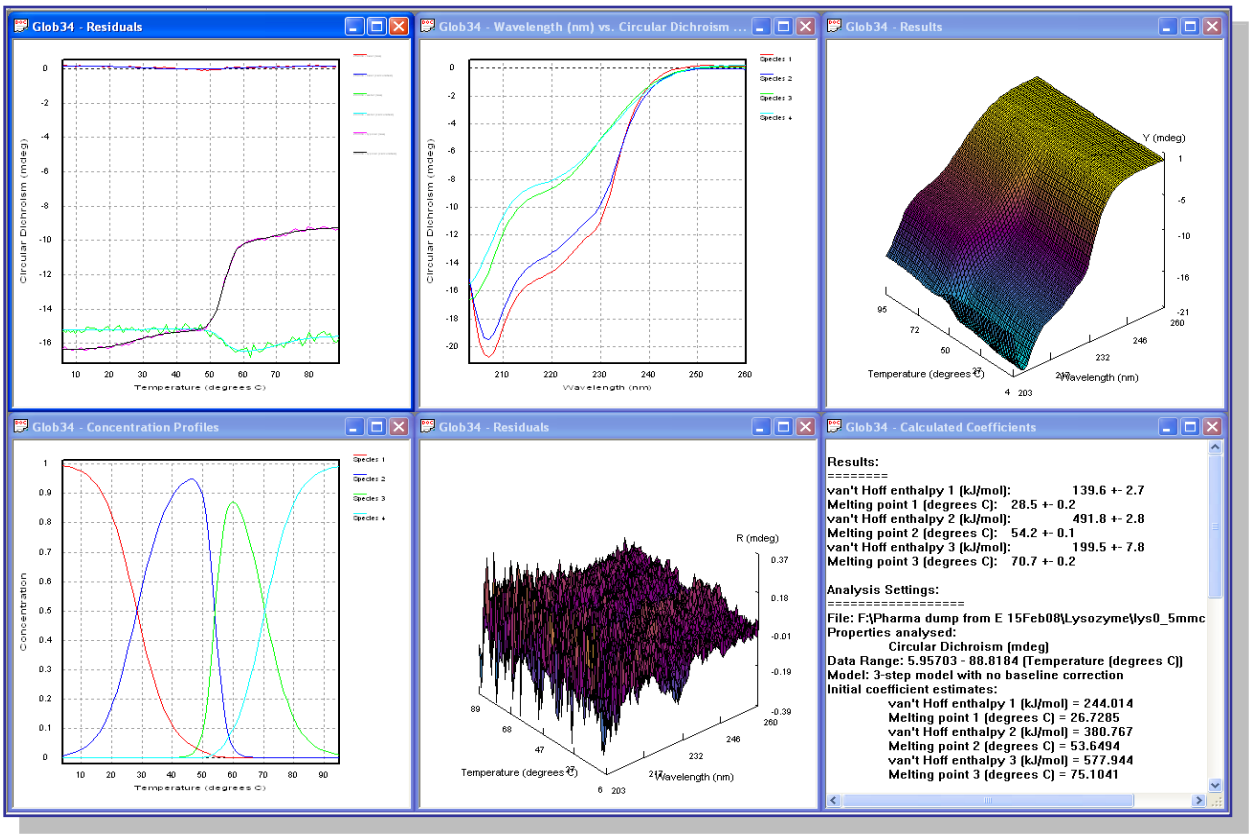
- The **CS/PCS Single Cell Peltier Temperature Controller** provides rapid and precise temperature control of the sample cell. The unit features a wide operating temperature range (-40°C to 105°C using a circulating chiller unit), excellent thermal contact and accommodates both rectangular and cylindrical and cuvettes. Other features include:

  - Temperature regulation precision: +/- 0.02°C
  - Temperature slew rate 10°C per minute
  - Magnetic stirring as standard
  - Customised cell temperature probes for direct monitoring of sample temperature
  - Total hardware control via Chirascan software
  - Demountable customised cell carriages, for rapid and convenient switching between cells of different dimensions and optimisation of heat transfer from block to sample
  - Optional cylindrical cuvette-holder available

  Suitable cells for far-UV and near-UV applications (listed above) are also included and are designed to maximise utility and minimise sample volume.

- The **GLOB3 global 3 analysis software** is a global analysis program specifically tailored for multi-wavelength spectroscopic data measured as a function of temperature. Typically, it is used to analyse protein CD spectra measured as a function of temperature. **Global 3** calculates the spectroscopic and concentration profiles of all the contributing species as well as the mid-points and van't Hoff enthalpies of the transitions between them. It thus delivers structural and thermodynamic data from a single experiment.

  Global 3 is an interactive program that is very intuitive and easy to use. Navigation within the program environment is straightforward and guidance is provided via a 'wizard'. In addition to the CD data, the corresponding absorbance/light scattering and fluorescence data can be readily accessed and used to decide on the fit limits in the wavelength and temperature domains. Principle component analysis is used to estimate the number of significant transitions present in the data prior to fit model selection.



- **CS/CFR 21 CFR Part 11 Compliance** CFR compliance is achieved through a combination of enforced administrative procedures, secure software, and secure data structures. Our implementation of 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.

A large range of other accessories are available (see pages 25-33) ensuring that **Chirascan-plus DMS** is also a versatile and future-proof CD spectrometer that can be adapted as research interests evolve.

Performance Specifications - Chirascan-plus DMS

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:	*< 0.02mº/hour*
CD & absorption detector:	*High-performance UV-Vis-IR avalanche photo-diode*
Wavelength range:	*Practical limits with sample in place: 170nm – 1150nm*
CD RMS Noise: *(All measurements at **1nm** BW and **2s** integration time)*	*0.03mº @ 175nm* *0.02mº @ 250nm* *0.02mº @ 180nm* *0.02mº @ 500nm* *0.01mº @ 185nm* *0.04mº @ 800nm* *0.02mº @ 200nm* *0.05mº @ 1000nm*
Wavelength scanning:	*Constant sampling & adaptive sampling stepped-scan*
Kinetic mode:	*Linear, logarithmic and split time-base. Up to 10,000 points per trace*
CD scale and resolution:	*±6000mº with automatic scaling.* *Resolution better than 0.001mº in 6000 mº*
Standard detection modes	
Spectroscopic probes:	***Simultaneous** circular dichroism, absorption,and fluorescence. Configurarable to FDCD.*
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*
Accessories included:	*Single-cell peltier, fluorescence detector and multi-channel detection card, rectangular cells: 4 of 0.5mm and 4 of 4 x 10mm with customised cell carriages, thermocouples: 2 of 0.25mm diameter, 1 of 1mm diameter.*
Software included:	*Pro-Data control & viewer software with **21 CFR Part II Compliance**, Global 3 global analysis software, CDNN secondary structure analysis software, APL data converter.*
PC interface:	*Windows 7*
Electrical Requirements:	*220/240, 110 V. 440 VA*
Available options:	*Multi-cell peltier, titrator, magnetic CD, fluorescence scanning monochromator, low temperature cryostat, fully integrated stopped-flow*

The applications notes summarised below are available on request.

Application note 1: Denaturation of a Monoclonal Antibody Under Different pH Conditions

In this study, DMS is applied to the denaturation of a monoclonal antibody under different pH conditions to show the potential of the technology in biotherapeutic development.

Introduction

The stability of a protein therapeutic is conventionally monitored by calorimetric techniques, particularly differential scanning calorimetry (DSC), yielding thermodynamic parameters such as the enthalpy and the mid-point of a conformational transition. The thermodynamic parameters are indicative of the relative stabilities of proteins in different conditions but calorimetric techniques cannot tell you how the conformation changes, whether or not a conformational change leads to aggregation, or if the protein is in its desired conformation prior to heating.

Knowledge of secondary structure will confirm whether or not the protein is correctly folded initially and observing the secondary structure as a function of temperature will tell you how its conformation changes on heating. However, the only common method to determine protein secondary structure in solution is circular dichroism (CD) and monitoring CD as a function of temperature can be a time-consuming business[1].

Chirascan dynamic multi-mode spectroscopy combines the benefits of spectroscopic and calorimetric measurements into a single, rapid, information-rich measurement that generates results from a complete experiment in about an hour.

Application note 2: Testing the Stability of Antibody Biotherapeutics in Different Formulations

For the series of experiments described here, DMS is used to investigate the stability of an antibody biotherapeutic in two different formulation buffers and to demonstrate the reproducibility of the calculated thermodynamic parameters T_m and $\Delta H_{van't\ Hoff}$. The technique used was dynamic multi-mode spectroscopy (DMS) and the work was carried out on a Chirascan-plus CD spectrometer.

Introduction

An interest had also been expressed in discovering whether DMS could identify any differences in the antibody denaturation signatures in the two formulations, following a request by the regulatory authorities to explain an apparent difference in T_m that had been highlighted by differential scanning calorimetry (DSC).

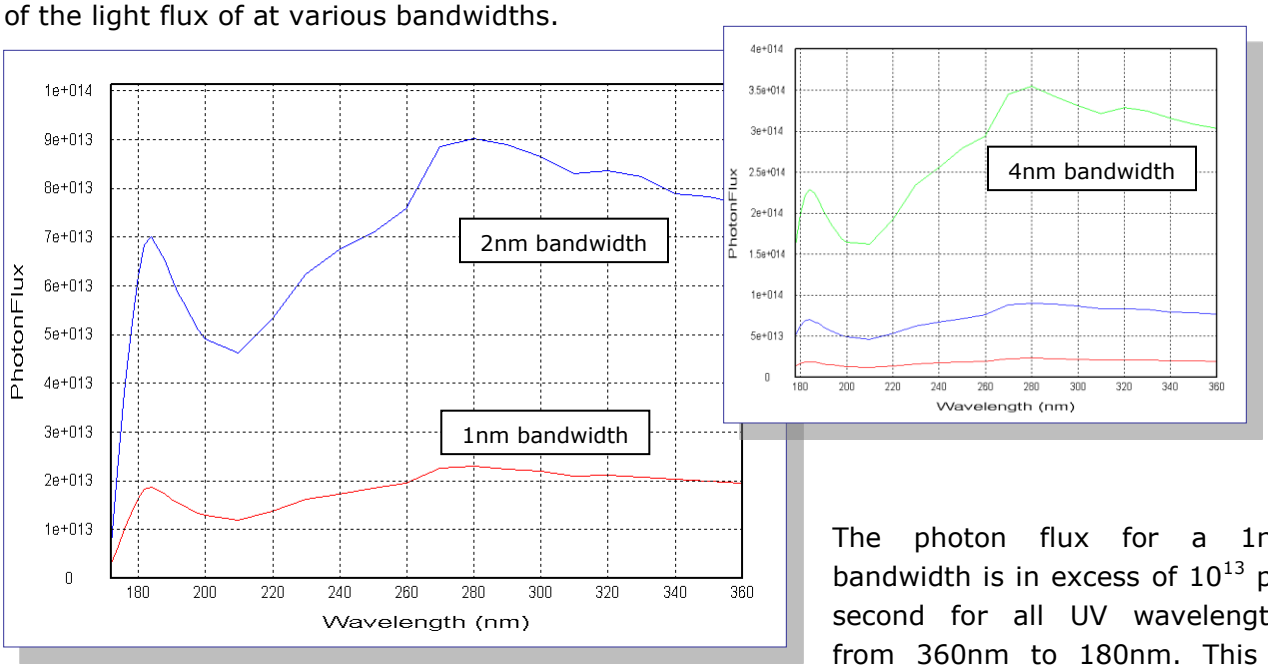
The samples were measured several times over a period of weeks and it became apparent that the antibody behaved differently as it aged in the two formulations, with lactate showing greater robustness than acetate.

[1]Norma J. Greenfield, Nature Protocols, Vol.1 No.6, 2006, 2527.

Performance Notes

Quality of Measurement and its Relation to Light Throughput

Chirascan and Chirascan-plus's high light throughput, particularly at far-UV wavelengths, is a key reason for its superior sensitivity and speed. Shown below are calibrated radiometric scans of the light flux of 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 as intense as 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 1nm is already 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 quality of measurement for a given measurement time or, equivalently, a measurement of a given quality can be completed much more quickly. Chirascan-plus has still greater performance benefits due to its more sensitive photodide detector (see page 6). Here are a few examples of why these benefits are important:

- In laboratories where there is high demand on the instrument, **productivity is significantly increased** without compromising data quality.
- In experiments that are demanding of sample, for example CD stopped-flow, the number of repeat measurements (and hence volume of sample) required to achieve acceptable quality is reduced. With ten times the light, **less than one-third of the sample** is required.
- 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 approximately one hour.

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

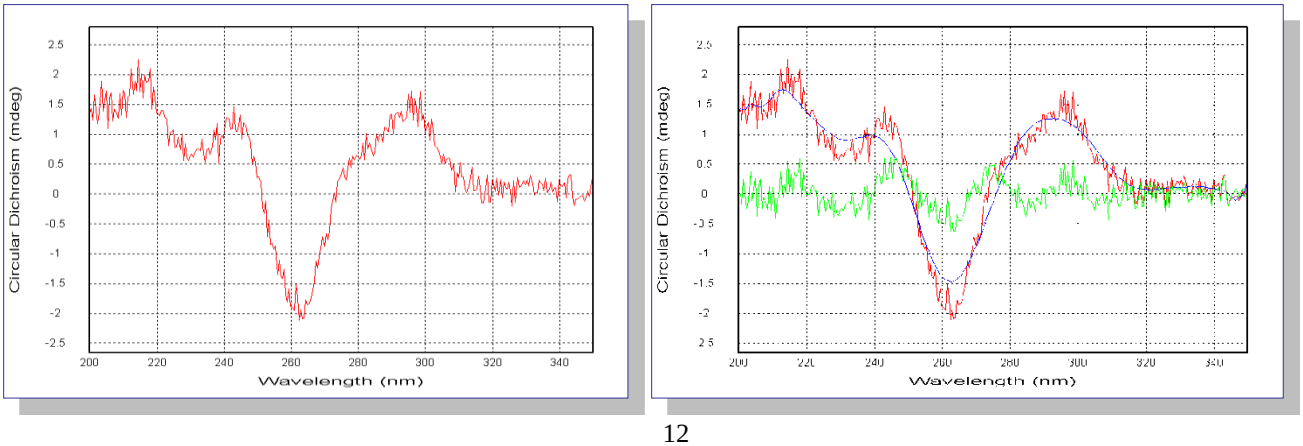
Because Chirascan has very high light throughput (see above), the raw, unsmoothed spectrum is of high quality and in general will need no further treatment. However, post-acquisition smoothing tools can be used to remove random noise elements if required. Two key advantages of this method of smoothing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

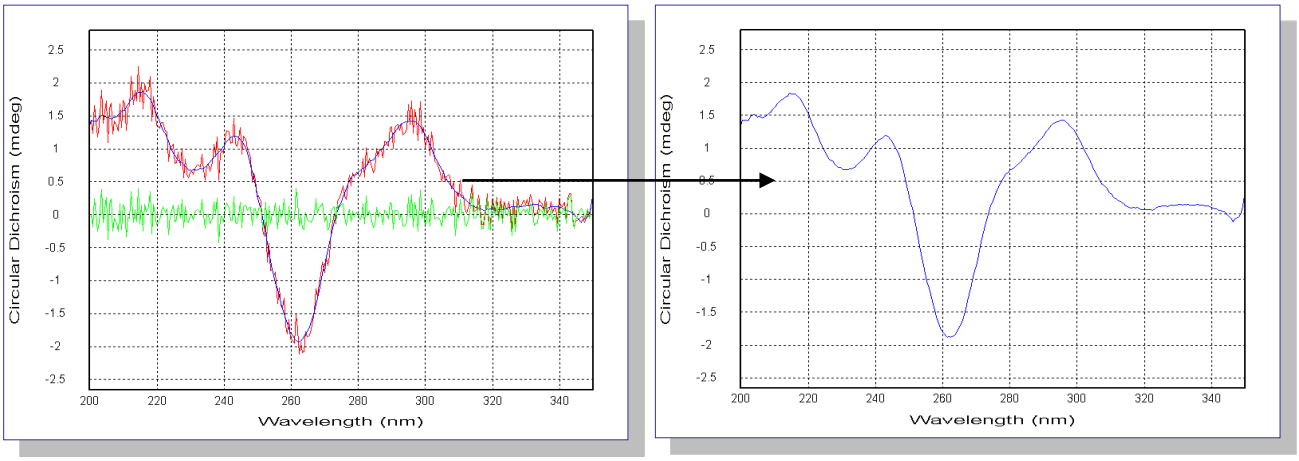
Of course data can be over-smoothed even when using post-acquisition filtering techniques (see section below) but this is always evident and can be corrected.

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

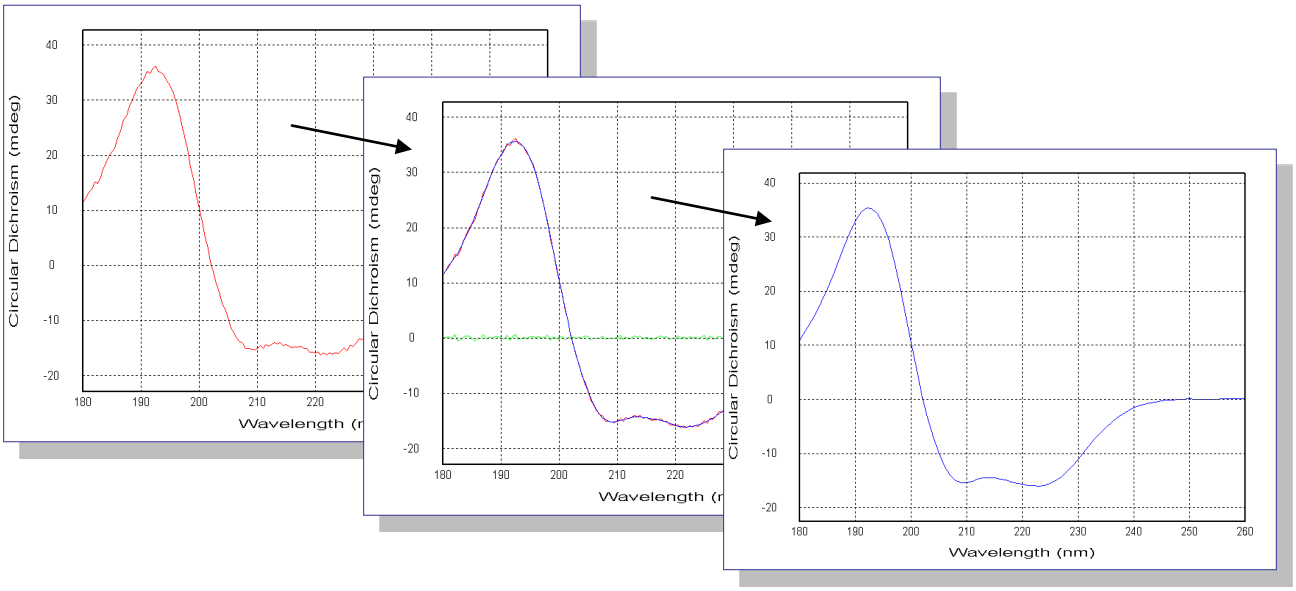


The CD spectrum shown above (left) was recorded on a Chirascan spectrometer and is raw (unsmoothed) data. In this example the spectrum is fairly noisy because of the small size of the CD signal and high scan speed used. If we now *over*-smooth this spectrum (above right), the resulting spectrum (blue 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.

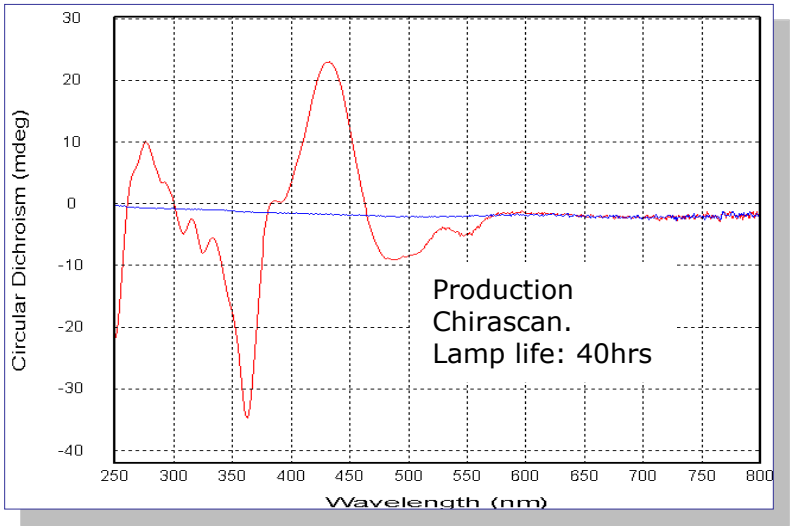
The second example (below) is more typical. The unsmoothed spectrum was acquired on a Chirascan spectrometer in 38 seconds and is of high quality due to Chirascan's high light throughput in the far-UV wavelength region. However some post-acquisition smoothing may be desirable to remove random noise elements.

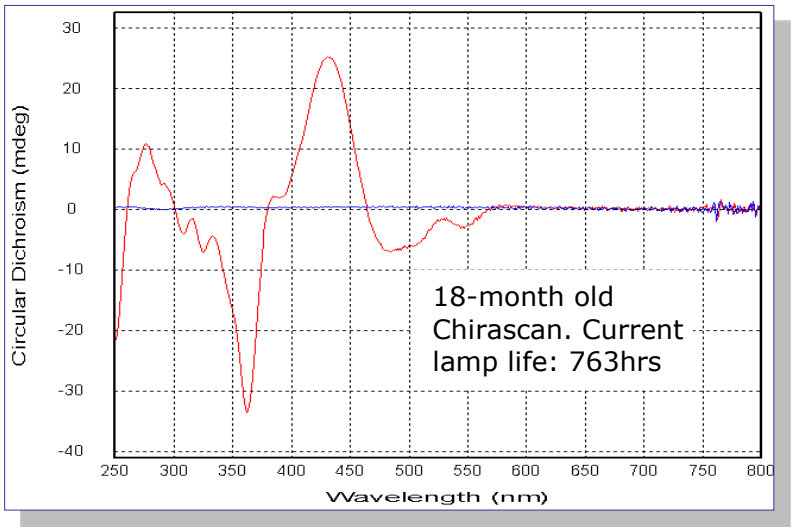


In summary, continuous scans use electronic filters to generate smooth CD spectra but may also produce distortion of the CD spectra. Stepped-scans record unfiltered CD measurements and so are always undistorted. If required, post-acquisition tools can be applied to smooth these spectra in a controlled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

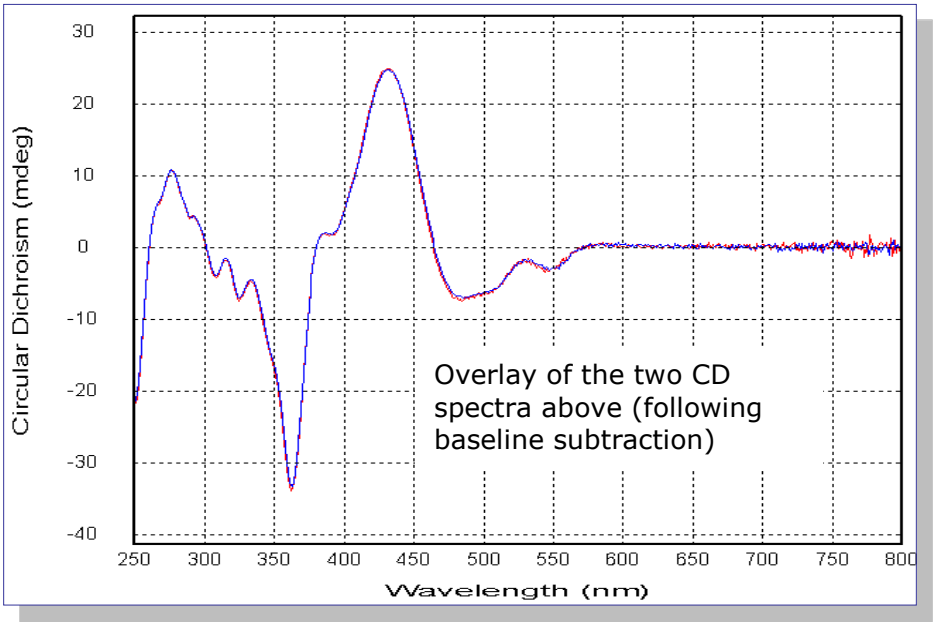
Long Term Stability and Accuracy

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
- remove oxygen from the light path (oxygen will absorb light at wavelengths in the far-UV)

Chirascan and Chirascan-plus require a total nitrogen flow of only 5 litres/min irrespective of the wavelength range being used (and only 2 litres/min when working above 200nm).

Chirascan and Chirascan-plus's sealed monochromator 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 rapid.

For the purpose of efficient nitrogen purging there are 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.

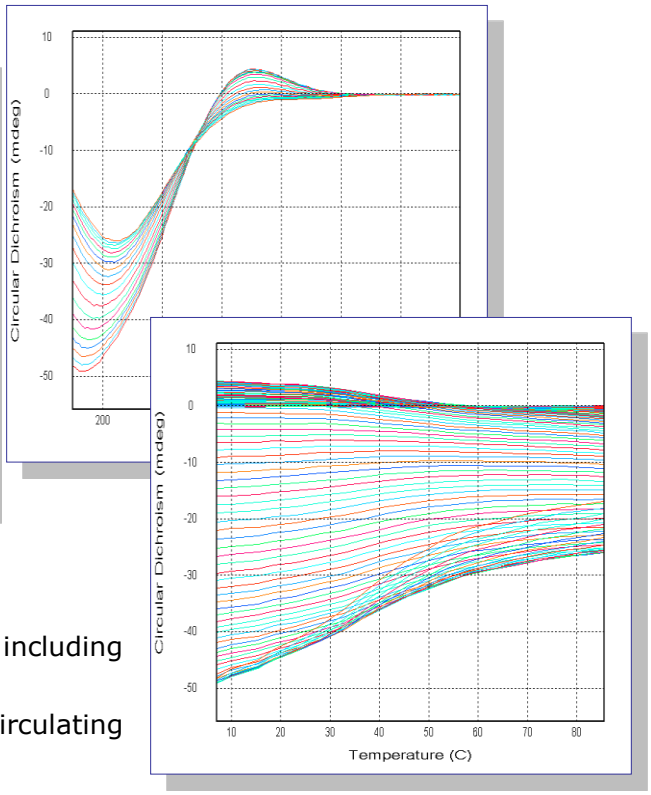
The Pro-Data Viewer software (for data display, manipulation and analysis) can be installed on an unlimited number of PC's for off-line data inspection.

An emulator version of Pro-Data 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.

Accessories and upgrades for new and existing systems

CS/PCM Four-Cell Auto-Changer with Peltier Temperature Controller

Chirascan-plus can be fitted with a 4-cell peltier temperature controller for rapid and precise temperature regulation of up to four samples. The **CS/PCM**'s turret design ensures that it is also suitable for fluorescence detection (and simultaneous CD and fluorescence detection). The 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 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.

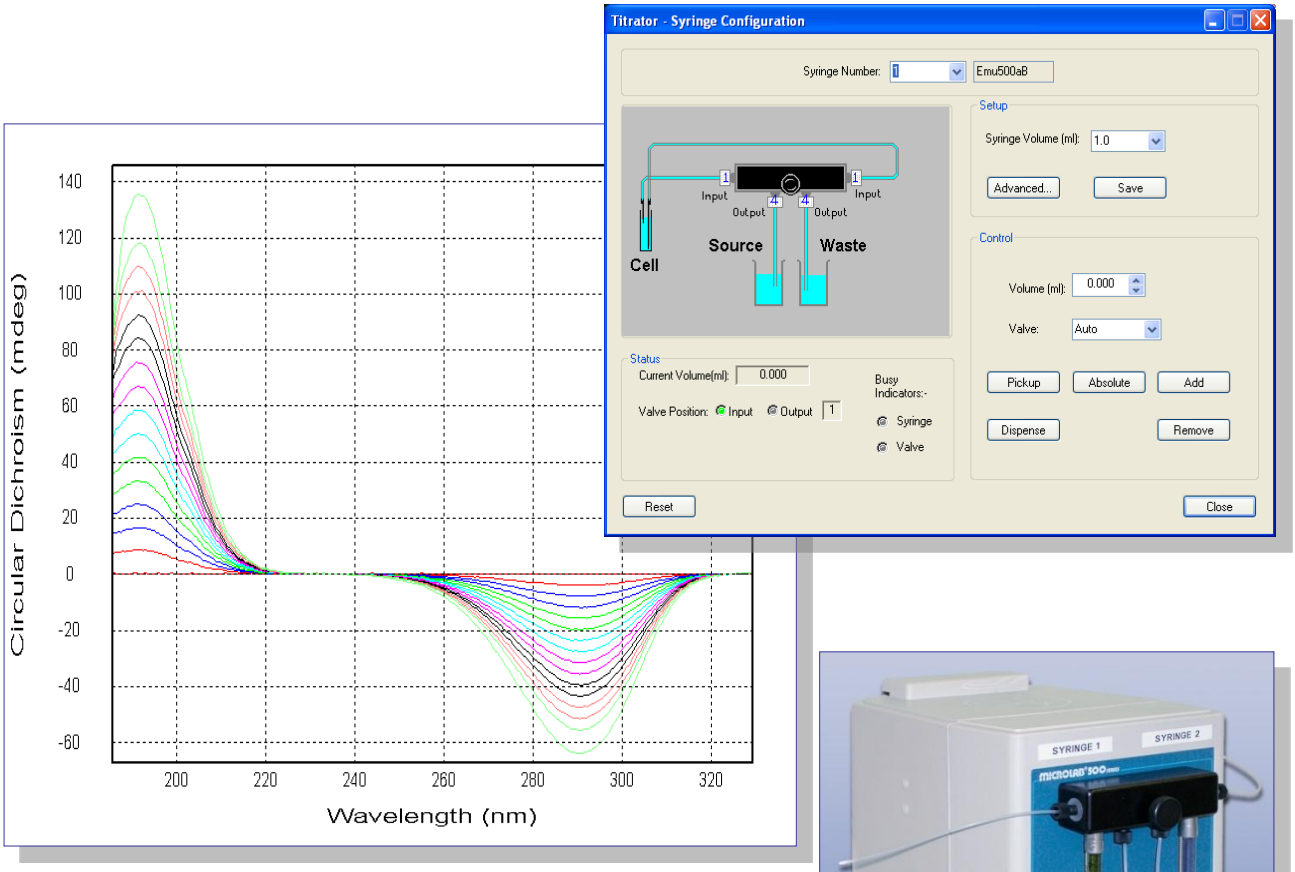


Accessory Features (CS/PCM)

- 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

CS/TT Dual-Syringe Titration Unit

The **CS/TT** 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 **CS/TT** 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 Pro-Data control software.

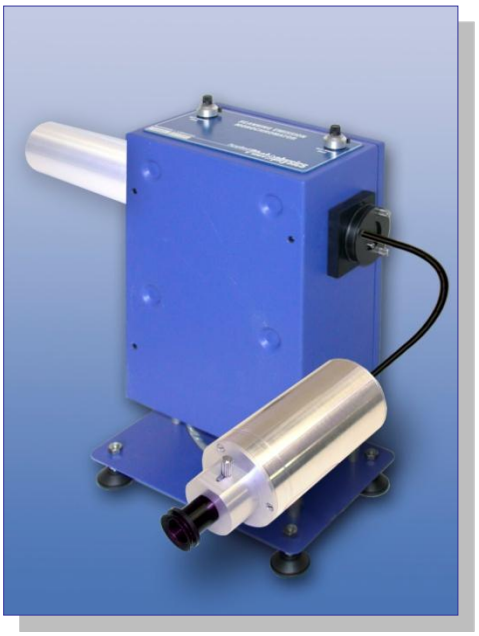


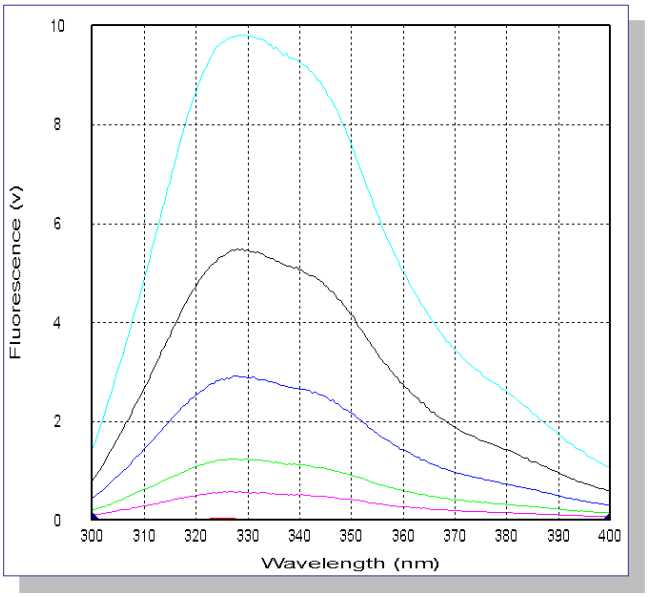
Accessory Features

- 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

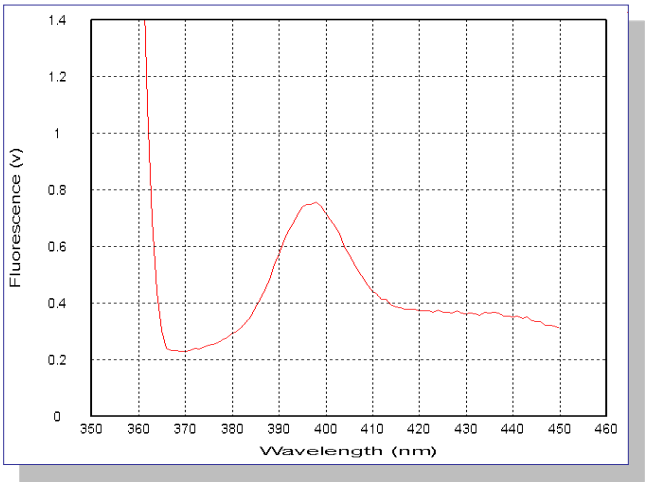
CS/SM Scanning Emission Monochromator

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





Fluorescence emission spectra recorded using the ***CS/SM*** *and* ***CS/TF*** *accessories.*



Fluorescence sensitivity is such that the Raman scattering of water is 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 ***CS/SM*** *and* ***CS/TF*** *accessories on a Chirascan spectrometer.*

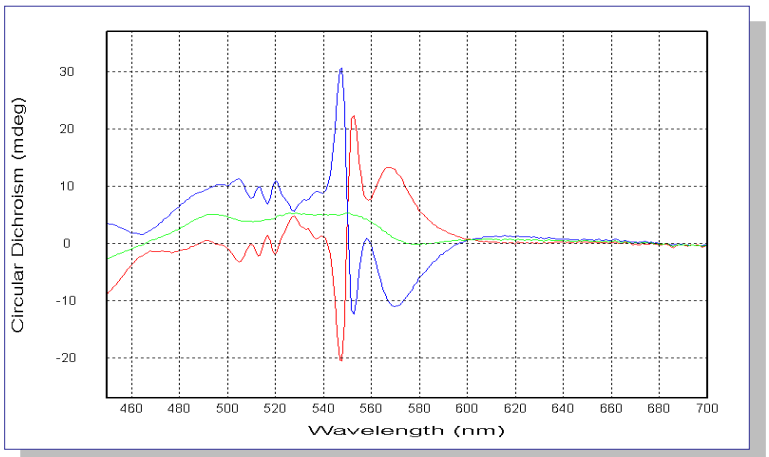
Accessory Features

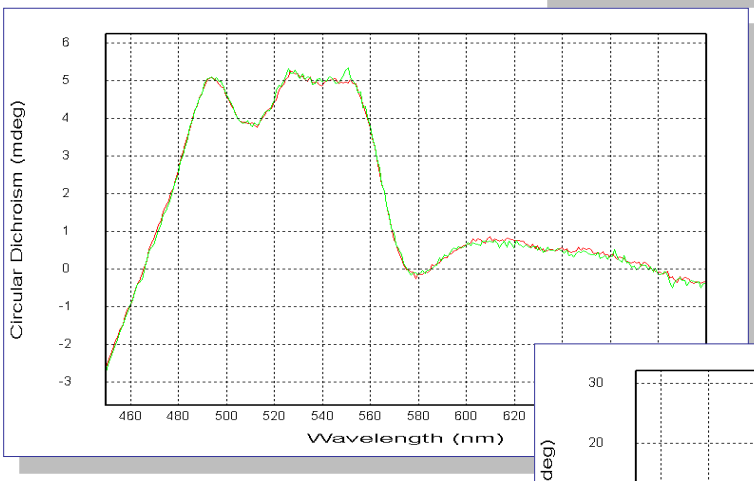
- Single diffraction grating symmetrical Czerny-Turner optical layout
- Fibre-optic light-guide coupling between cuvette and monochromator entrance
- Full software control of scanning experiments
- May be operated using the standard CD channel or a dedicated fluorescence channel (**option CS/MC**) which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

CS/MCD Magnetic Circular Dichroism

The **CS/MCD** accessory mounts directly into Chirascan-plus'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 > 0.95 Tesla.

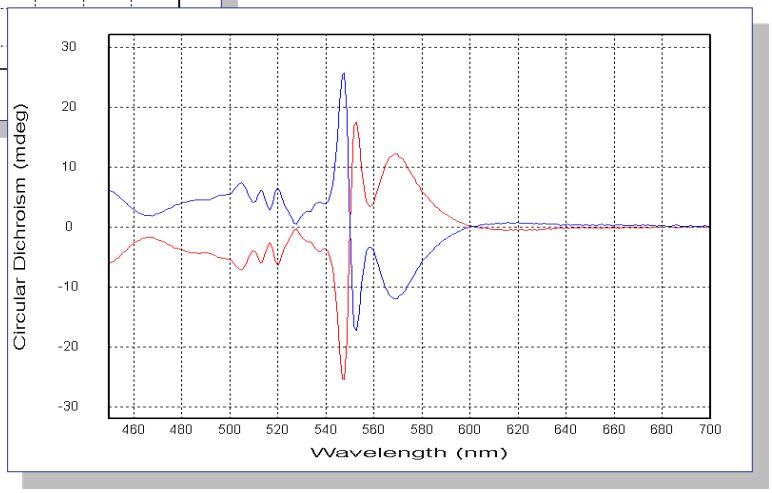
The spectra (right) show MCD spectra of Cyctrochrome C (blue and red traces) collected on a Chirascan instrument. The scans are raw MCD data collected in 7 minutes - no smoothing (or filtering) has been applied. The average of these spectra (green trace) should in theory be the non-magnetized CD spectrum 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 capability is included as standard. FDCD can be recorded using the standard Chirascan or Chirascan-plus instrument after relocating the (CD) detector to the side observation port used for fluorescence measurements.

J100 Julabo AWC-100 recirculating cooler for use with the peltier

The peltier accessories **CS/PCS** and **CS/PCM** require water circulation for cooling. The **J100** 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 Litres
Dimensions (W x L x H): 20.5 x 36 x 31.5 cm
Weight: 11 kg

CS/LT Nitrogen Cryostat Accessory

Chirascan-plus 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 **CS/LT** 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.

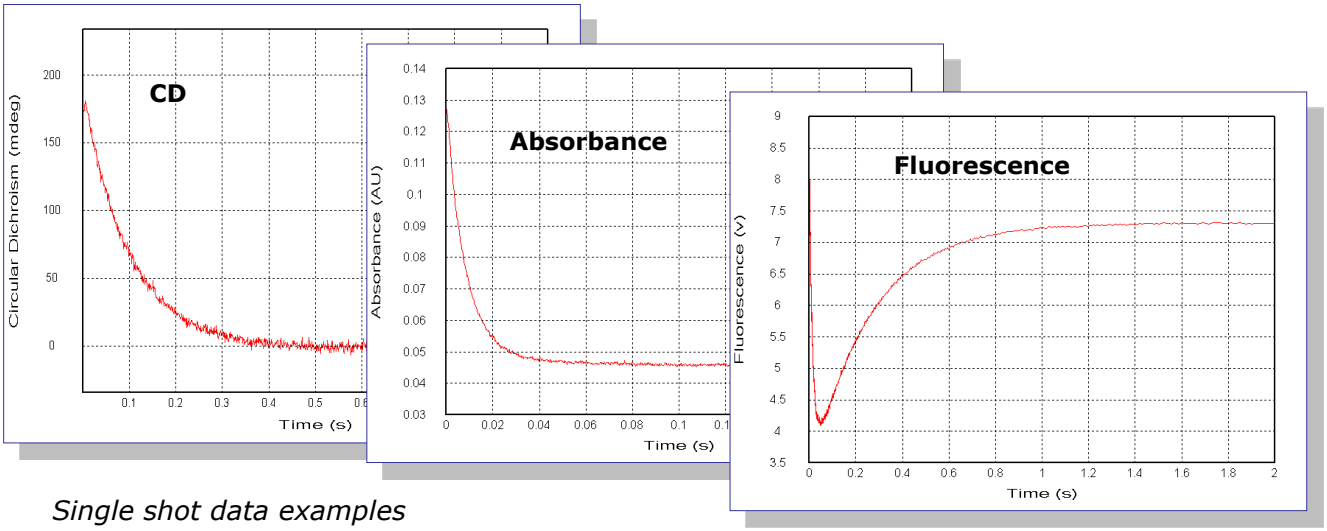
Accessory Features

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

CS/SF Stopped-Flow Unit

The **CS/SF** accessory brings to 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 **CS/TF** detector option.



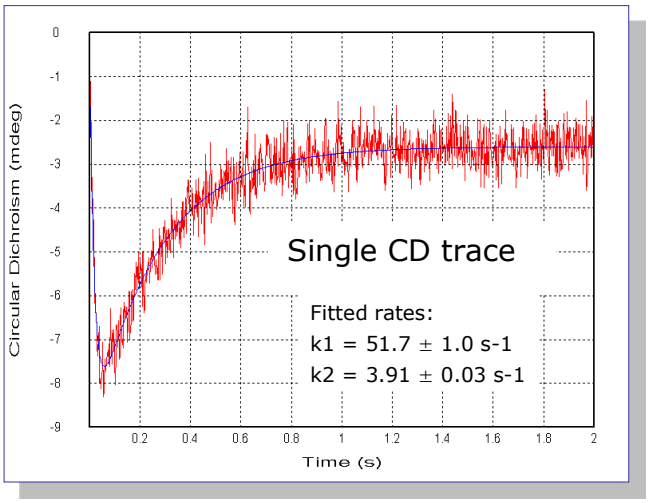
Single shot data examples

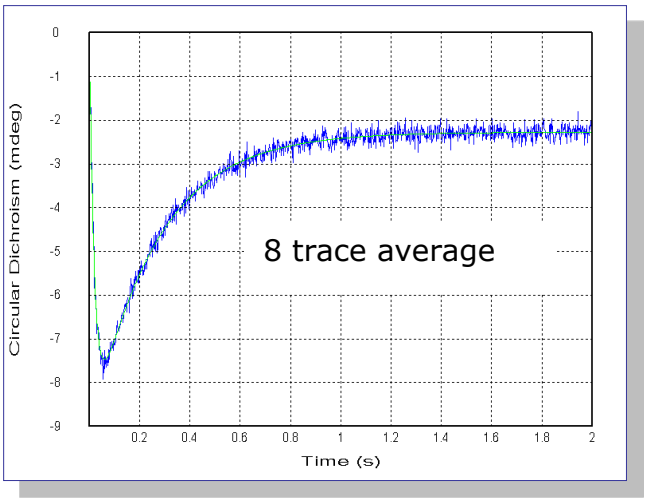
Accessory Features

- 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

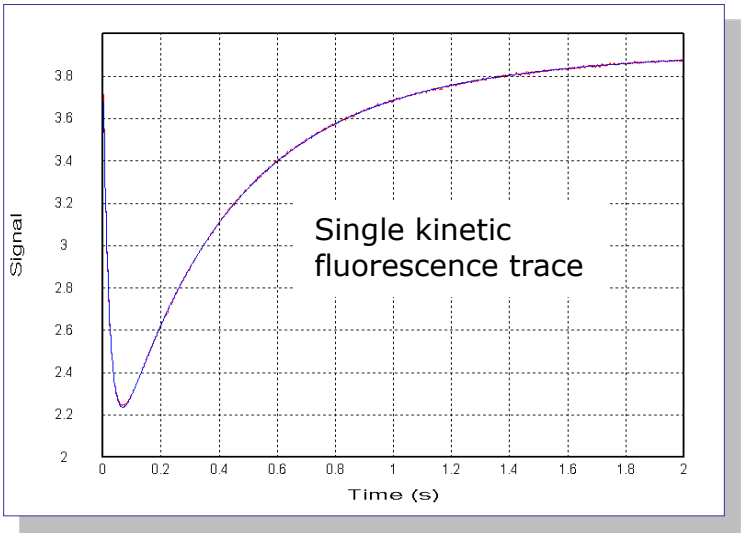
- Comprehensive range of accessories available including sequential-mixing, fluorescence polarisation and dual fluorescence detection.
  The example below, recorded on Chirascan-plus with the **CS/SF** accessory, shows the refolding of lysozyme from 6M GuHCl by a 1 in 10 dilution in buffer. Below left 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 75 traces to obtain equivalent data quality to the single kinetic trace shown above.



The stopped-flow fluorescence trace shown above (using the **CS/TF** fluorescence detector) 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.

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