JASCO

J-810 Spectropolarimeter

Circular Dichroism Chiroptical Spectrometer

Leading the development of ch

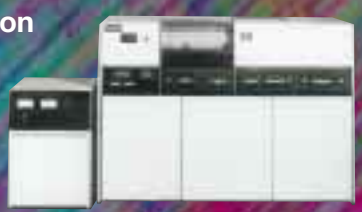
Early in the nineteenth century, scientists including Biot started to study the optical characteristics of quartz and organic solutions. By 1817, work on Optical Rotatory Dispersion (ORD) had been published. During the next 150 years, key discoveries included:

- 1845 Magnetic optical rotation - Faraday
- 1860 Molecular asymmetry and antipode - Pasteur
- 1866 Optical rotation measurement (Na - D line) - Bunsen
- 1874 Optical activity and the stereoisomerism of molecules - Van't Hoff and LeBel
- 1895 CD and ORD Cotton effect - Cotton
- 1911 Coordination theory of metal complexes - Werner
- 1959 Moscowitz Kronig-Kramers relationship (ORD/CD) - Moffit
- 1960 Djerassi applies ORD to the determination of the absolute configuration of steroids.
- 1961 Moffit, Woodward, Moscowitz, Klyne and Djerassi publish their Octant rule covering the configuration of cyclohexanone rings.

At this time JASCO saw the requirement for chiroptical spectrometers to study stereochemistry. Since launching the AP-1 in 1961, JASCO has been the leading manufacturer of Spectropolarimeters for measuring Circular Dichroism (CD) and Optical Rotatory Dispersion (ORD). The development of these chiroptical spectrometers has furthered the study of optically active substances. Now, the determination of absolute molecular configuration, changes of conformation and stereochemical analysis have become rapid, routine procedures.

1961 AP-1
the first JASCO spectropolarimeter (ORD)

1965 ORD-UV-5
with CD option



1970 J-20
ORD/CD
spectropolarimeter

1972 J-40
first dedicated CD unit

1978 J-500
data pro
updated

iroptical instrumentation

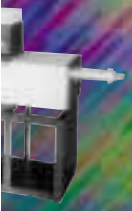
1999 J-810
the latest CD development from JASCO

1995 J-715
updated J-710 with enhanced specification

1990 J710/720
next generation CD spectropolarimeter followed by J-730 for NIR applications

1986 J-600
fully PC controlled CD

1980 J-200
NIR CD unit (800-2000nm) spectropolarimeter



cessing CD with electronics

The J-810 Spectropolarimeter

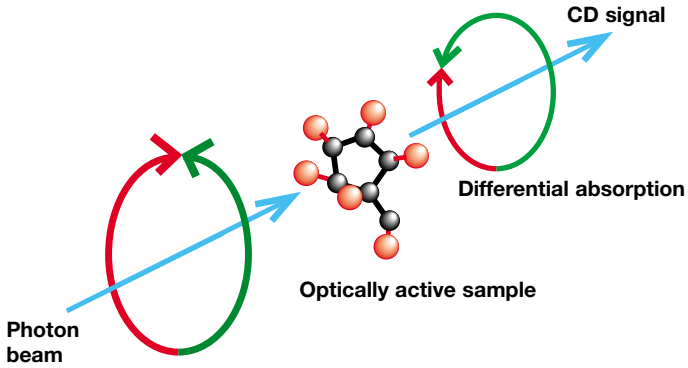
A CD spectropolarimeter is a hybrid instrument consisting of a variable wavelength polarimeter and absorption spectrophotometer.

Although the technique was 'invented' by Cotton in 1896, it was only in France during 1960 that it came to the forefront of analytical research.

The potential of Optical Rotatory Dispersion (ORD) had been acknowledged by instrument manufacturers and CD accessories were developed to compliment the ORD technique for detecting chiral compounds.

The J-810 CD spectropolarimeter is the latest in a long line of innovative spectroscopic instruments from JASCO during the past forty years.

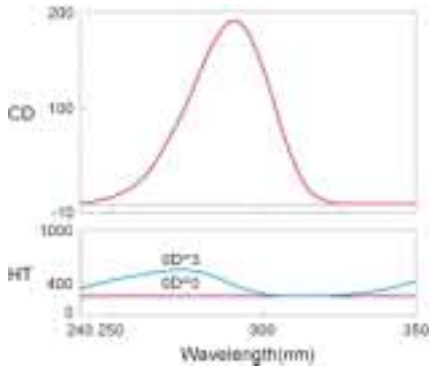
It offers an even better working specification plus a wider range of accessories for an enhanced list of measurement techniques.



CD is the difference in absorption coefficients, of an optically active sample, for left and right circularly polarized light.

System specification

- Greater wavelength range
  163-900nm (standard)
  163-1100nm (with optional NIR sensitive PMT)
- Increased stability
  ± 0.03 mdeg/hour
- Excellent (S/N ratio) sensitivity
  0.035mdeg @ 200nm
  0.045mdeg @ 185nm



The J-810 is equipped with an extremely low straylight (0.0003% or less) monochromator enabling CD spectra of highly absorbing samples to be obtained. The CD data of NH_4-d-10 Camphorsulphonate does not distort, even when a highly absorbing sample is placed into the optical path of the spectropolarimeter.

Key features

- Four channel simultaneous data acquisition giving multi-angle analysis of sample
  - Select from two internally generated signals: CD, FDCD, ORD, LD, UV, Fluorescence, Temperature and two external channels (eg. pH)

- 3 scanning modes
  Continuous:
  - Running average method

  Step:
  - Fixed wavelength method for sample scanning

  Auto response:
  - Based on the stepscan method but response changes automatically with absorbance

- Real time autoscaling minimizes pre-analysis set up

- New Spectra Manager™ Software
  - Macro command
  - JASCO canvas publishing software

- New measurement techniques
  - Fluorescence (F)
  - Circularly Polarized Luminescence (CPL)
  - Double beam UV

- Improved mechanical design
  - Compact with built in air cooled light source and power supply

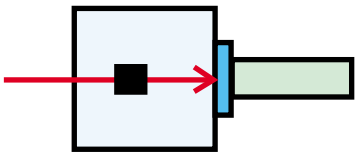
Validation software

Validation of analytical instrumentation is a key factor in today's laboratory.

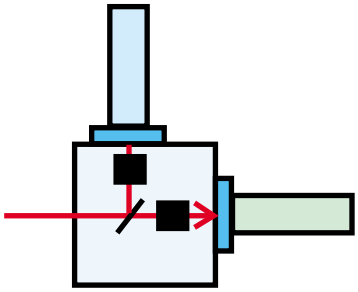
This program is used to set up and monitor a range of tests to check the performance of the J-810 spectropolarimeter. Each test follows a JASCO standard operating procedure (SOP).

Modes of operation

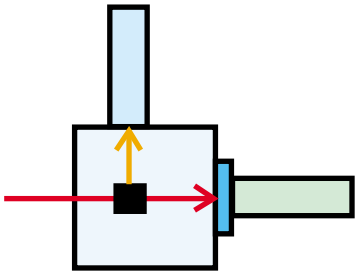
CD



Absorbance



Fluorescence/CD



Measurement systems and hyp

Circular Dichroism (CD)

The standard measurement technique of the J-810 is the collection of Circular Dichroism spectra.

Auto-Titration

It is possible to carry out titration experiments directly in a standard cell using a dedicated microtitrator. This is important for monitoring the denaturation of proteins and ligand binding experiments.

Stopped-Flow CD (SFCD)

The primary objective of this technique is the study of fast chemical and biological reactions. By using a stopped-flow device in conjunction with the CD spectrometer, transient changes in chiroptical properties associated with chemical, biochemical and biophysical reactions, can be investigated. Stopped-flow absorbance and fluorescence measurements can also be made.

Chiral HPLC Detection (LCCD)

Selective detection of optically active compounds gives confirmation of enantiomeric separation and determination of elution order. Isomeric purity can also be calculated.

Magnetic Circular Dichroism (MCD)

This is Circular Dichroism induced by the presence of a magnetic field parallel to the light path of the spectropolarimeter. MCD reflects the electron state (particularly spin state) of molecules and atoms.

Fluorescence Detected CD (FDCD)

The difference in fluorescence intensity for left and right circularly polarised excitation is measured. The detector is placed 90° from the lightpath. The technique is very selective for fluorophores, even in multichromophoric molecules at microdetection levels.

phenated techniques

The world's first Circularly Polarized Luminescence (CPL)

When chiral compounds are excited by natural light, there is a difference between the right and left circular polarization intensities of fluorescence and phosphorescence. This phenomena is CPL. Normal CD measurements give information about chirality of ground state molecules. CPL gives details of chirality at excited states. It is used for fluorescent chiral organic compounds, chiral complexes of rare earth metals and proteins.

Optical Rotatory Dispersion (ORD)

The ORD technique provides information on molecules without chromophores such as saccharides, and compliments standard CD measurements. It determines the absolute configuration of non-absorbing substances.

Linear Dichroism (LD)

A technique used widely for measuring achiral (and occasionally chiral) molecules in anisotropic solutions.

Near Infrared CD (NIRCD)

Metals linked with proteins and chiral metal complexes give CD active bands in the NIR region. The working range of the J-810 covers applications in this region.

Double Beam UV

The acquisition of high resolution spectrophotometric data.

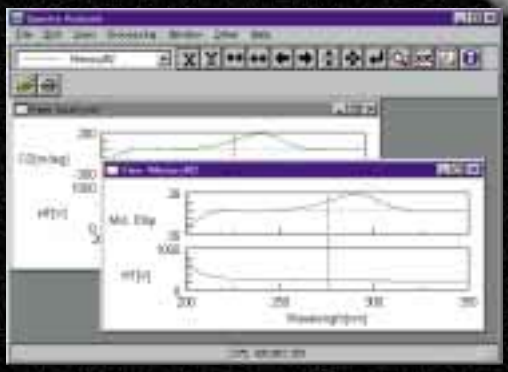
Fluorescence Scanning with EM Monochromator

Simultaneous collection of CD and fluorescence data. Fluorescence scanning can be combined with titration and thermal ramping capability giving two sets of experimental data from a single sample.

Total Fluorescence (TF)

A technique using two detectors (one positioned at 90° to the lightpath) that enables simultaneous collection of CD and fluorescence data. A technique growing in popularity for thermal melt experiments monitoring conformational changes.

Spectra Manager™



JASCO is the first manufacturer to offer a powerful 32 bit Windows software platform for controlling a range of spectroscopic instrumentation and analyzing the experimental data.

J-810 hardware system
System configuration and spectrum measurement parameters are easily set up within the Spectra Manager™ package. Self diagnostic routines continuously monitor the performance of the spectropolarimeter.

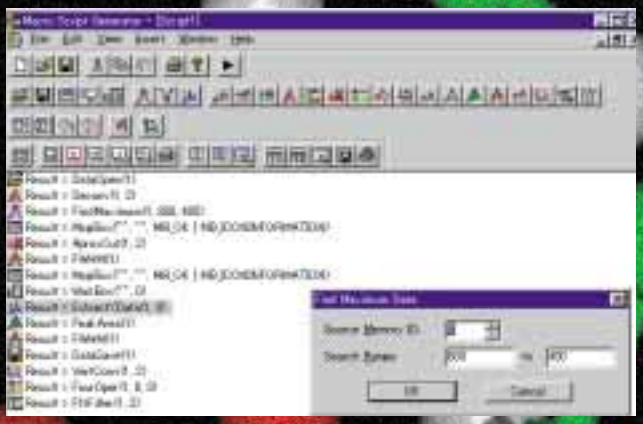
System monitoring
Four channels of data can be aquired simultaneously. Standard specifications include CD/HT and CD/Absorbance modes but other signals including CD/Fluorescence, temperature and pH can be measured for specific application requirements.

Data analysis
The spectrum analysis program is a comprehensive package for capturing and processing spectral data. Features include:

- Spectrum display and overlay
- Add/subtract/divide
- Baseline correction and x-y axis conversion
- Derivatives
- Peak height/area/half-value width computation
- Smoothing/enlargement/reduction
- Peak detection
- Data conversion (JCAMP-DX and text format)
- File search with graphics or parameters

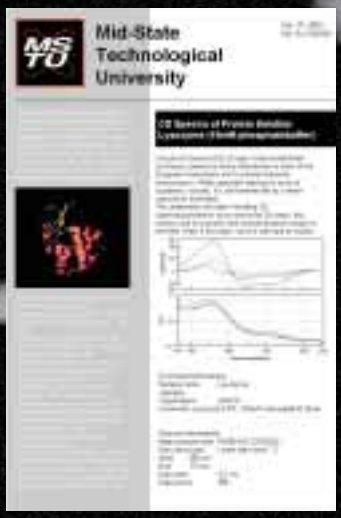
Customized data publishing
JASCO canvas provides a means for the user to produce publication quality layouts of data text, graphics, photographs and logos to meet their own report requirements.

Macro command software

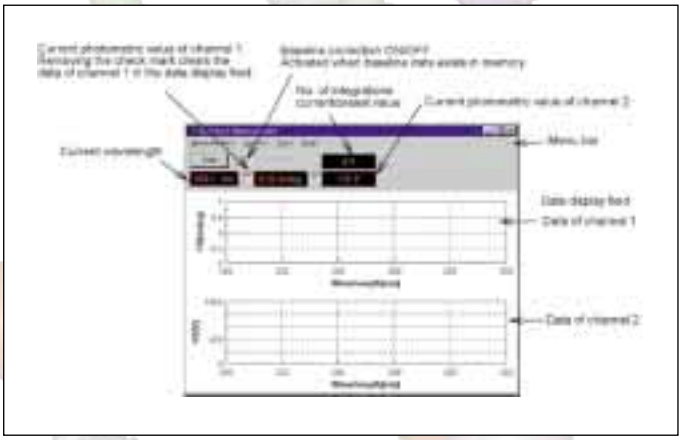


This program automates a complete range of tasks during an analytical run including the change of experimental parameters and the control of system accessories. These include:

- scan wavelength (performs spectral measurement under chosen parameters)
- scan time (time variation measurement)
- scan temperature (temperature measurement)
- loop (repeats the number of times for the specified macro command range)
- wait (waits a set time until executing next command)
- set temperature
- increment temperature (by chosen value)
- move wavelength (to new experimental wavelength)
- increment wavelength (by chosen value)
- dispense titrator steps (by set volume from syringe)



JASCO canvas



System monitoring

Software programs

Secondary structure analysis of protein
Uses the reference CD spectra of Professor Jen Tsi Yang, University of California, as the basis for the estimation. The program executes secondary structure estimation and calculates structural components including helix, beta, turn and random coil.
Information includes the overlaid CD spectrum of protein, CD spectrum of protein being calculated and residue CD spectrum. (Figure 1) Other secondary structure algorithms are available.

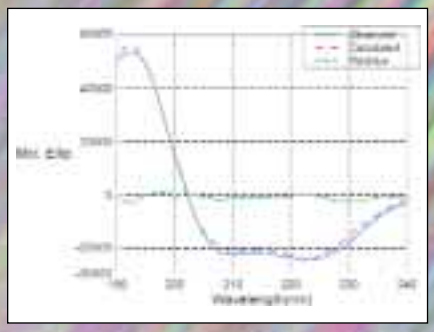


Figure 1

Denatured protein analysis
The thermodynamic parameters of a protein are calculated from variable temperature data.

Temperature control
Temperature control programs enable higher order structural data of proteins to be determined. CD spectra obtained at a preset temperature can be overlaid or displayed in 3D format. Figures 2 and 3 show CD temperature variation for Lysozyme and a temperature scan CD for the same molecule.
The latter, using data obtained at 220nm, indicates a dramatic change in secondary structure around 70°C.

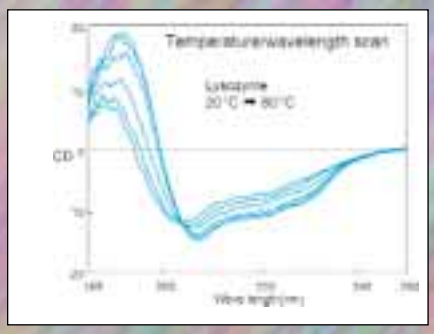


Figure 2

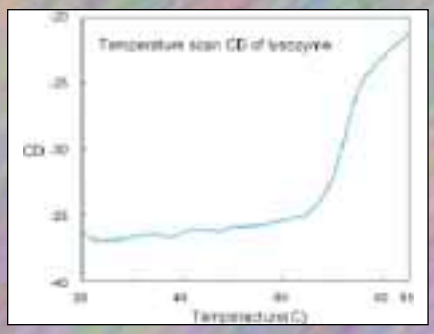


Figure 3

Multi-wavelength variable temperature measurement program
A peltier type control system or bath circulator plus thermostatted cell holder are used in conjunction with this program to monitor CD changes at a maximum of 8 wavelengths.

Curve fitting analysis
Used to find heights, widths and positions of multiple overlapping bands in a spectrum. (Figure 4)

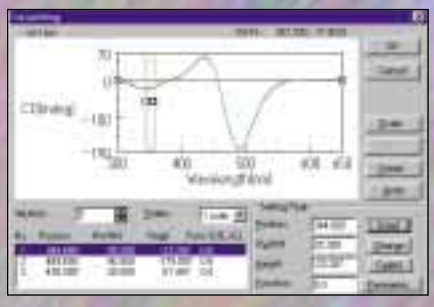


Figure 4

g-factor software
The g-factor is an indicator of optical purity. It is calculated by dividing the measured CD value by the total absorbance of the sample. It provides a simple means of determining the isomeric purity of samples under evaluation. (Figure 5)

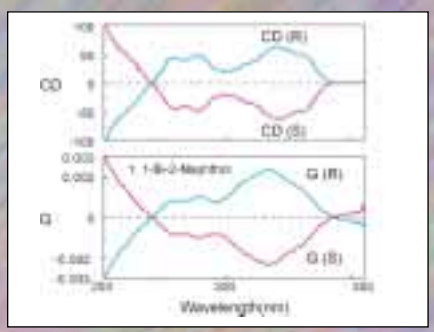


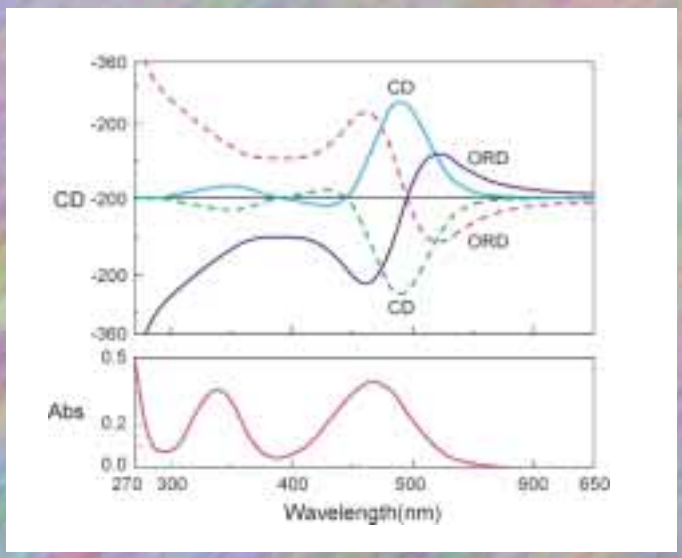
Figure 5

Applications

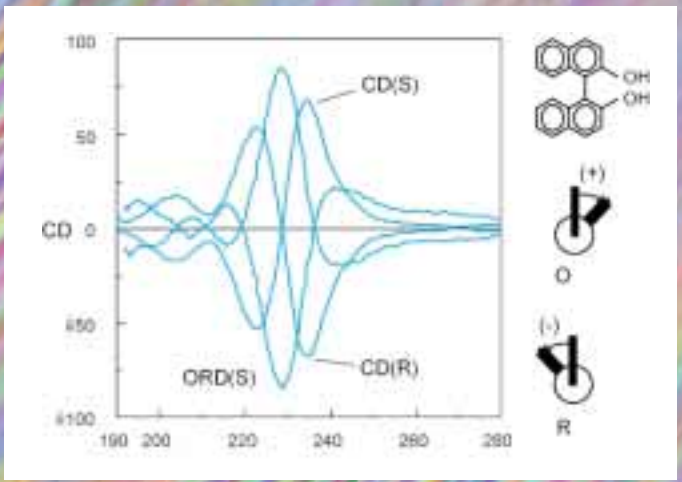
CD/ORD instruments are now used extensively in a number of application areas:

- Protein folding studies
- Protein conformational studies
- DNA/RNA interactions
- Enzyme kinetics
- Organic stereochemistry studies
- Purity testing of optically active substances
- Quantitative analysis of pharmaceuticals
- Natural organic chemistry
- Biochemistry and macromolecules
- Metal complex chemistry
- Polymer chemistry
- Medical science
- Agrochemistry
- Physical chemistry
- Rapid scanning (time resolved) experiments

Lysozyme
Normally proteins exist in a state where basic secondary structures such as alpha-helixes and beta sheets are folded (beta-turn), forming unique tertiary structures.



Metal complex chemistry
CD and ORD are used to analyse chiral metal complexes such as tris-ethylenediamine cobalt.



Natural organic and pharmaceutical chemistry
CD and ORD assist the identification of compound chirality. The example shows spectra of a (R)- and (S)-1, 1-Bi-2 naphthol derivative giving clear information about the molecular chirality.

Applications

CD
Used for a wide range of applications from UV analysis through to the Near-infrared region.

Far-UV (250-170nm)
Secondary structure analysis of Polypeptide and protein. Protein tertiary structure classes. Figure 6 shows the CD spectrum of D-Camphor.

Near-UV (350-250nm)
The ultraviolet CD, around 280nm, is caused by tryptophan and tyrosine residue within the tertiary structure of Lysozyme. Information from this area correlates protein structure with activity. Below 250nm, a peptide bond (amide group) is the chromophore. The spectrum (Figure 7) reflects secondary structures such as alpha-helixes and beta sheets. As an analytical technique, CD offers the best means of observing secondary structures of molecules.

Near-UV to Near-IR (300-1000nm)
Near infrared is effective for coloured proteins. Figure 8 is the CD spectrum of copper gluconate.

ORD
Effective for samples without chromophoric groups such as saccharides. It complements CD in the analysis of chiral compounds and the determination of functional groups.
The UV and CD response of glucose lie at wavelengths less than 200nm. Figures 9 and 10 show these spectra. At 185nm, the absorbance due to water can mask the CD response of glucose, so the ORD technique is used to follow the mutarotation of glucose.

CPL
Normal CD and UV absorbance spectra give chirality information for the ground state of molecules (Figure 11). The low energy electronic state of Camphor results in the absorption of light. CPL gives chirality information for the excited state of molecules. With molecules in an excited state, chromophoric groups emit light with a different content of left-handed and right-handed circularly polarized light and drop to a ground state. (Figure 12)

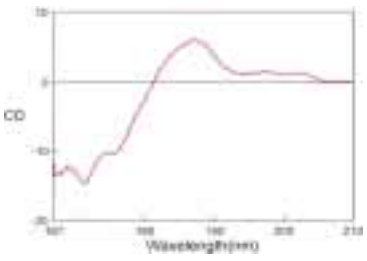


Figure 6

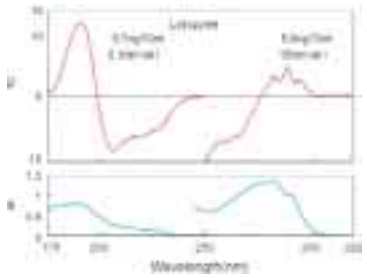


Figure 7

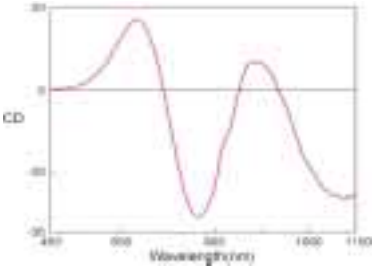


Figure 8

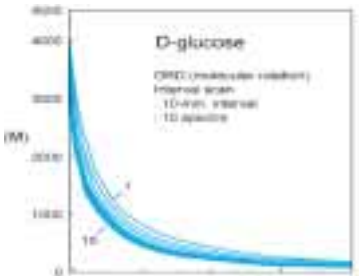


Figure 9

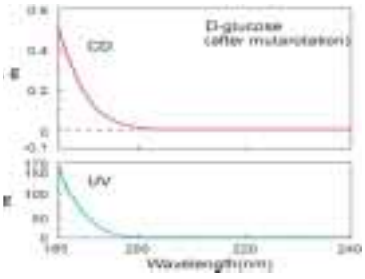


Figure 10

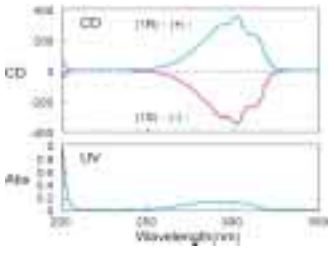


Figure 11

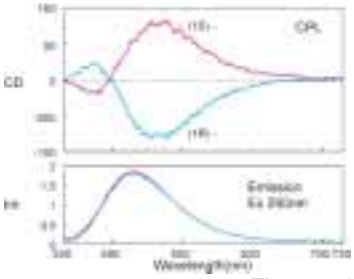


Figure 12

MCD
Circular Dichroism information evoked by the magnetic field gives electron state (spin) details of molecules and atoms. (Figure 13)

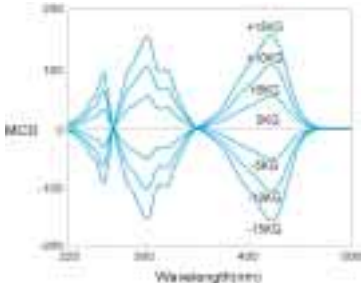


Figure 13

FDCD
This method of detecting CD uses fluorescent light. It is particularly effective for protein analysis and chirality detection in polymeric materials.

The spectrum of 1(S),2(S) -t- cyclohexanediol bis (6- methoxy -2- naphthoate) is an example of the high sensitivity given by the FDCD technique (Figure 14). When a molecule contains a fluorescent chromophoric and a non-fluorescent chromophoric group, the CD of the former is often not detected. It is hidden by the stronger CD of the latter group. FDCD selectively captures the CD of fluorescent chromophores.

Figure 15 shows the selective FDCD of a tryptophan residue that is normally hidden by the CD of human serum albumin.

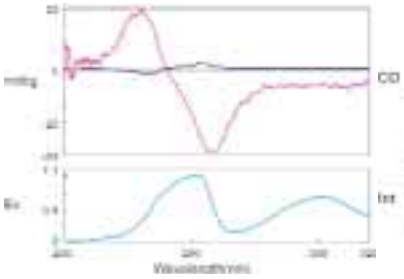


Figure 14

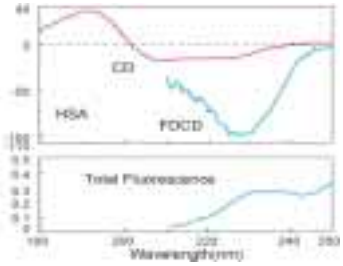


Figure 15

Micro-analysis (Microcell)
For low volume (100µl) CD measurement with cylindrical cells (having low distortion property) and rectangular microcells. (Figure 16)

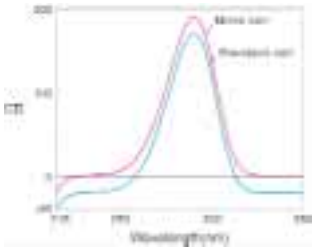


Figure 16

Fluorescence/CD
The multi-channel J-810 allows total fluorescence measurements to be made while monitoring routine CD data.

Figure 17 shows CD/Fluorescence data for Lysozyme which was taken simultaneously with the CD/HT data.

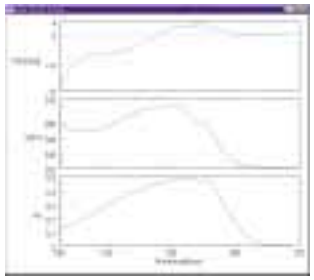


Figure 17

Accessories

Rapid kinetics (stopped-flow CD) system

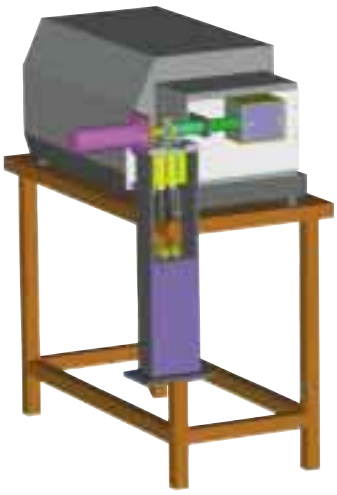
The JASCO Model J-810 CD spectrometer is coupled with the Bio-Logic stopped-flow modules providing high speed mixing for the study of kinetics and protein folding in both absorbance and fluorescence modes. The Bio-Logic stopped-flow modules can be equipped with either 2, 3 or 4 syringes which are controlled by stepping-motors enabling extremely precise delivery and millisecond dead time mixing. In addition, the Bio-Logic stopped-flow modules can be equipped with a titration accessory allowing the SFM to be used as a fully programmable titration unit.

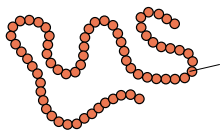
Rapid kinetics (protein refolding) monitored by using stopped-flow/CD/Fluorescence

Refolding measurement of Cytochrome C
Cytochrome C in its unfolded state, denatured in the presence of guanidine hydrochloride, is refolded by dilution of the guanidine hydrochloride with a sodium phosphate buffer. This refolding process, which is completed in around 300msec, is monitored by simultaneous CD/Fluorescence measurement with stopped flow dilution.

Kinetic trace at 222nm (secondary structure region)
Figure 18 gives CD and Fluorescence spectra of Cytochrome c, showing the unfolded and refolded states, in the secondary structure wavelength region. A change in this region (225nm) is largely due to alpha-helical content. Figure 19 shows CD and Fluorescence kinetic traces at 220nm when Cytochrome C in guanidine hydrochloride (unfolded state) was mixed with sodium phosphate buffer using the Biologic uSFM-20 two syringe microvolume stopped-flow and JASCO CD/Fluorescence simultaneous measurement attachment.

Kinetic trace at 289nm (aromatic side chain region)
Figure 20 gives CD and Fluorescence spectra of Cytochrome c, showing the unfolded and refolded states, in the near UV (aromatic side chain) region. Changes in this region reflect changes in the local environment of aromatic side chains and tryptophan residues. Figure 21 shows CD and Fluorescence kinetic traces at 289nm. Cytochrome C is refolded in a mixture of guanidine hydrochloride and a sodium phosphate buffer.

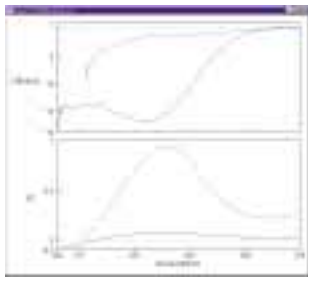




Primary protein structure - Amino acid chain

alpha-helix

Secondary protein structure - Sequence of Amino acids linked by hydrogen bonds



Unfolding state (random)
Refolding state (alpha-helix)

Figure 18

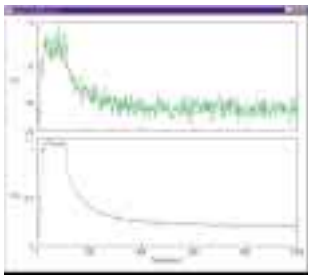
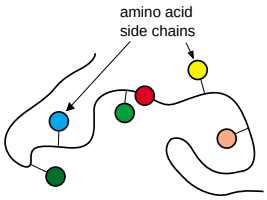
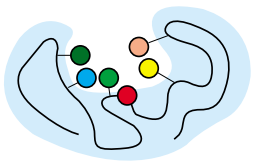


Figure 19

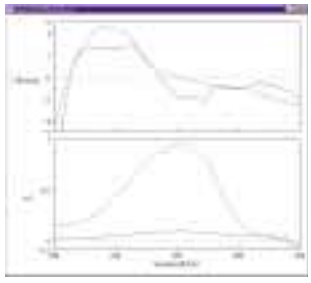


unfolded protein

UNFOLDING ↑↓ FOLDING



folded protein



Unfolding state
Refolding state

Figure 20

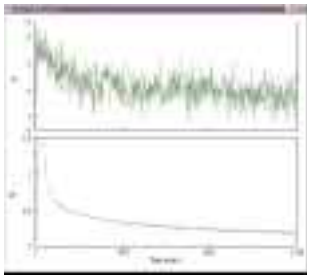
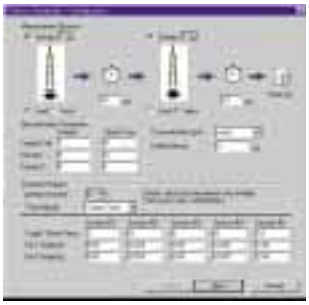


Figure 21

Automatic Titration Measurement



Titration Parameter Configuration

Titrant Table

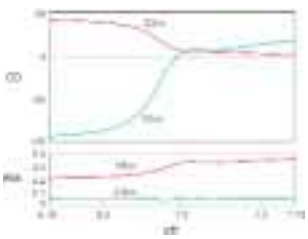


Figure 22

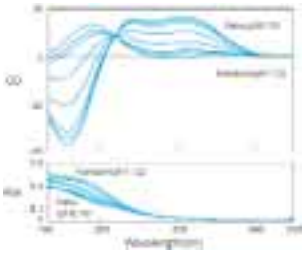


Figure 23

Automated-titration system

The system is designed to automatically monitor changes in the CD, Abs (HT), Fluorescence, pH and other parameters while injecting and withdrawing solutions from the cuvette. Dual syringes are employed, each equipped with a valve to allow automated refilling/flushing during extended runs. Two titration modes are provided;

Volume mode:
where the user selects the titrant volume per step and number of steps.

Concentration mode:
where the user selects a target concentration per step and the volume per step is automatically computed.

At the end of a run the raw data is displayed. This is subject to concentration change due to the titration. An auto-correction mode compensates for the concentration change and displays the true signal to titrant dependency.

Secondary structural change of synthetic polypeptide by pH change
The carboxyl groups of the side chain of poly-d-glutamic acid have negative charges in a solution of pH7.1, resulting in a random coil structure. Following a pH change toward the acidic side, the negatively charged carboxyl groups are protonated resulting in a change to alpha-helical structure.

In this experiment, 0.25mM sulfuric acid was added (2.5µl per step, up to 300µl) to 2ml of 0.001% sodium poly-d-glutamate (NaPDG) solution (pH7.01). The pH and CD were measured at 222nm. In addition, a CD spectrum was measured at every 25µl addition of sulfuric acid in the range from 250 to 186nm. Figure 22 shows the CD intensity change at fixed wavelength under the same pH conditions. Figure 23 shows the CD and UV spectra of NaPDG by pH change from 7.01 to 5.78.

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