

CD spectrum measurement using high-throughput CD

Introduction

Automatic measurement systems have been drawing increasing attention among pharmaceutical companies due to their ability to analyse multiple samples automatically, saving both time and man power and eliminating any possible human error. Since CD spectroscopy allows the user to easily obtain structural information about proteins and peptides, automated measurement systems can be used to optimize the screening process of a large number of samples.

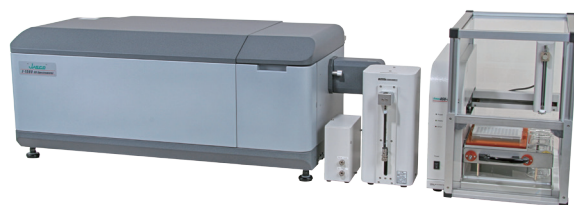
This application notes introduces the High-Throughput CD system for automatic CD measurements of multiple protein samples.

Keywords

J-1500, circular dichroism, Autosampler, ASU-800, FMO-427, Emission monochromator, excitation, emission, High-Throughput, HTCD, proteins, biochemistry, pharmaceuticals

Experimental

Measurement conditions			
Data acquisition interval	0.1 nm	Response time	2 seconds
Spectral bandwidth	1 nm	Scan speed	100 nm/min
Accumulations	4 times	Path length	1 mm
Emission wavelength	350 nm	Sensitivity	850 V



JASCO J-1500 high-throughput CD system
View product information at www.jascoinc.com

Results

First the sequence of operation and data acquisition must be defined in advance. The maximum number of samples that can be analyzed automatically is 192. The system will then carry out CD measurements automatically and save the data series. The following sequence is automatically performed by the HTCD system:

1. Loading of measurement condition
2. Baseline correction
3. Sample 1 measurement (cytochrome c)
4. Washing
5. Sample 2 measurement (lysozyme)
6. Washing
7. Sample 3 measurement (human serum albumin)
8. Washing
9. Sample 4 measurement (hemoglobin)
10. Washing

	Type	Vial No.	Flush	Bubbling	Sample Name	Conc. [(w/v)%]	Comment	File Nam
1	Load Parameters		<input type="checkbox"/>	<input type="checkbox"/>				C:\Y_¥Para-4.cdsp
2	Baseline	5	<input checked="" type="checkbox"/>	<input type="checkbox"/>				
3	Sample	1	<input checked="" type="checkbox"/>	<input type="checkbox"/>	CytC	0.01	CD/FL_1mm	C:\Y_¥2011_10_19~
4	Sample	2	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Lyz	0.01	CD/FL_1mm	C:\Y_¥2011_10_19~
5	Sample	3	<input checked="" type="checkbox"/>	<input type="checkbox"/>	HSA	0.01	CD/FL_1mm	C:\Y_¥2011_10_19~
6	Sample	4	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Hb	0.01	CD/FL_1mm	C:\Y_¥2011_10_19~
7			<input type="checkbox"/>	<input type="checkbox"/>				
8			<input type="checkbox"/>	<input type="checkbox"/>				
9			<input type="checkbox"/>	<input type="checkbox"/>				
10			<input type="checkbox"/>	<input type="checkbox"/>				
11			<input type="checkbox"/>	<input type="checkbox"/>				
12			<input type="checkbox"/>	<input type="checkbox"/>				

Figure 1. Display of sequence setting

The JFLC-499 CD/emission flow cell was used to obtain both CD and excitation spectra. The results of the automatic measurements of CD, absorbance, and excitation spectra for four 0.1 mg/mL protein samples are shown below. These high speed measurements took a total of 3 minutes per sample (each scan is 45 seconds and there are 4 accumulations).

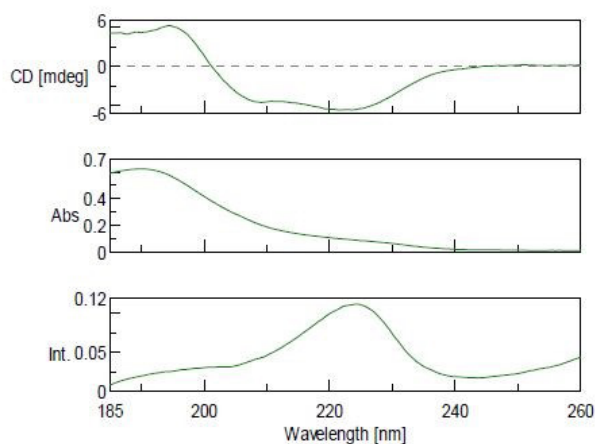


Figure 2. CD (top), absorbance (middle), and excitation (bottom) spectra of cytochrome c.

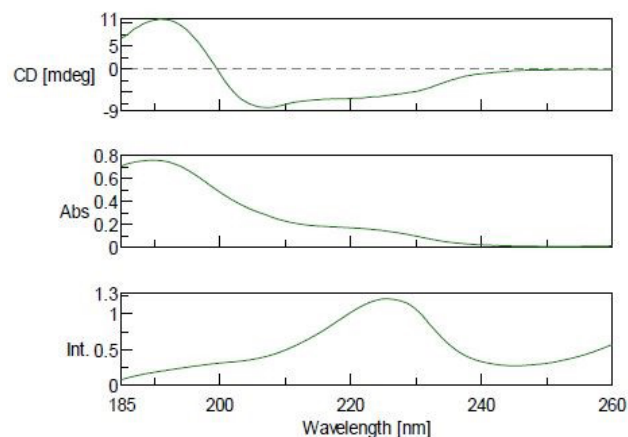


Figure 3. CD (top), absorbance (middle), and excitation (bottom) spectra of lysozyme.

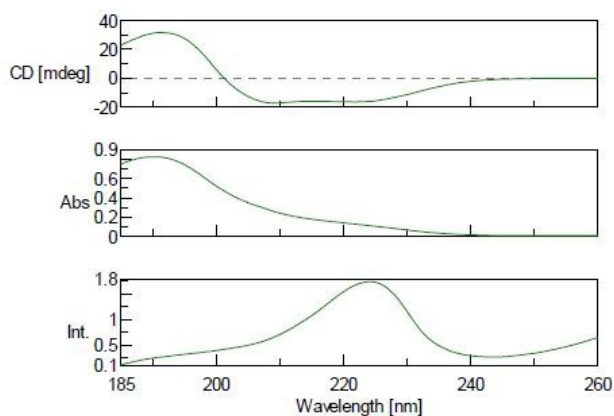


Figure 4. CD (top), absorbance (middle), and excitation (bottom) spectra of human serum albumin.

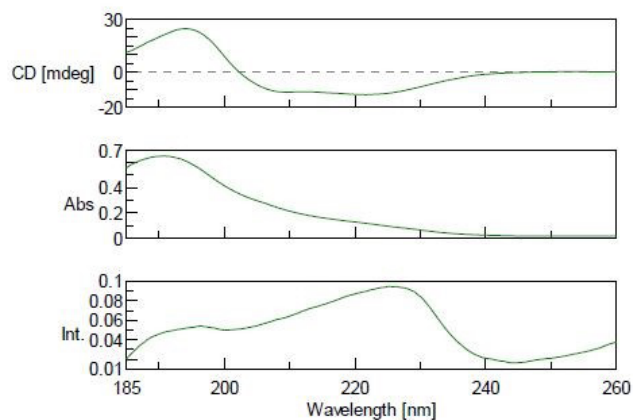


Figure 5. CD (top), absorbance (middle), and excitation (bottom) spectra of hemoglobin.

Conclusion

This application note demonstrates that high quality data can be measured for three different spectroscopy techniques (CD, absorption, and excitation) in a matter of minutes. The J-1500 coupled with the HTCD system allows over 100 samples to be measured automatically without human operation, saving time and eliminating human error.