

CD Spectrum measurement using High-Throughput Circular Dichroism (HTCD) system

Introduction

Automatic measurement systems of multiple samples have been drawing attention among pharmaceutical market. Since CD spectroscopy is useful to obtain the structural information of protein and peptide rather easily, it could be utilized in the optimal system for screening of multiple samples, which is related to drug discovery handling lead compounds and seed compounds.

In this paper, automatic CD spectrum measurement of multiple proteins using HTCD system is introduced.



Fig. 1 HTCD system

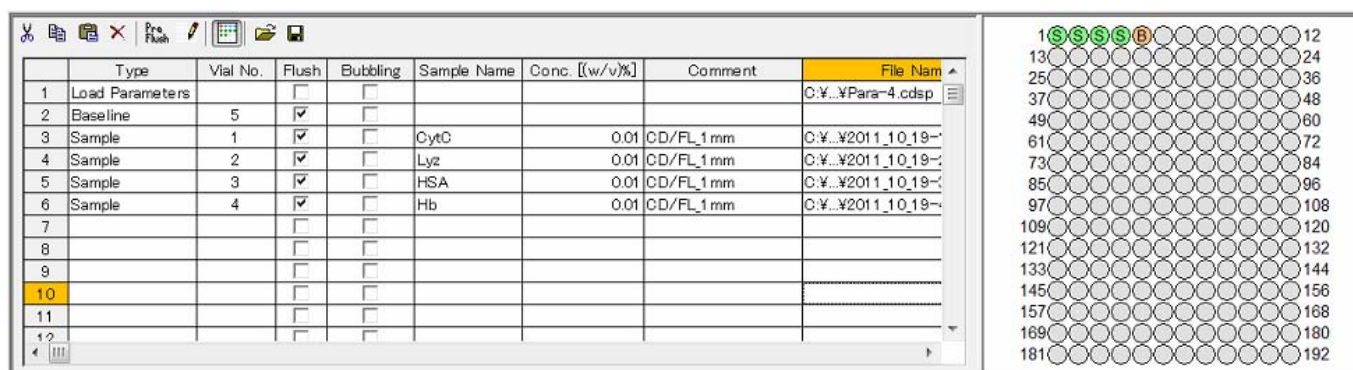
The dedicated software which supports this sampling system and automatic measurement could be an effective tool for this automation.

Setting of sequence

Since the operation of sampling sequence and data acquisition is defined in advance, CD measurement is automatically carried out and the results are automatically saved as a series of data set. The optimal measurement condition can be pre-determined depending on the sample.

(Sequence)

1. Loading of measurement condition	6. Washing
2. Baseline correction	7. Sample measurement (human serum albumin)
3. Sample measurement (cytochrome c)	8. Washing
4. Washing	9. Sample measurement (hemoglobin)
5. Sample measurement (lysozyme)	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:\...\Para-4.cdsp
2	Baseline	<input checked="" type="checkbox"/>	<input type="checkbox"/>				
3	Sample	<input checked="" type="checkbox"/>	<input type="checkbox"/>	CytC	0.01	CD/FL_1 mm	C:\...\2011_10_19-
4	Sample	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Lyz	0.01	CD/FL_1 mm	C:\...\2011_10_19-
5	Sample	<input checked="" type="checkbox"/>	<input type="checkbox"/>	HSA	0.01	CD/FL_1 mm	C:\...\2011_10_19-
6	Sample	<input checked="" type="checkbox"/>	<input type="checkbox"/>	Hb	0.01	CD/FL_1 mm	C:\...\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"/>				

Fig. 2 Display of sequence setting

Result

Maximum 192 samples can be analyzed automatically without human operation according to the preset sequence including the steps from measurement to results saving.

Utilizing JFLC-499 CD/emission flow cell enables to measure not only CD/Abs. spectra but also excitation and emission spectra. The results of automatic measurement of CD/Abs./excitation spectra of 4 kinds of proteins are shown as below. High speed measurement such as 3 minutes for each sample (1 scan 45 seconds x 4 times accumulation) was achieved and quite high quality CD/Abs./excitation spectra were obtained.

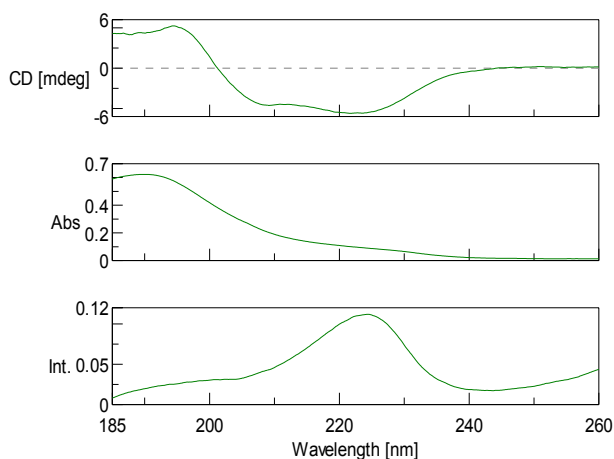


Fig. 3 Spectra of cytochrome c

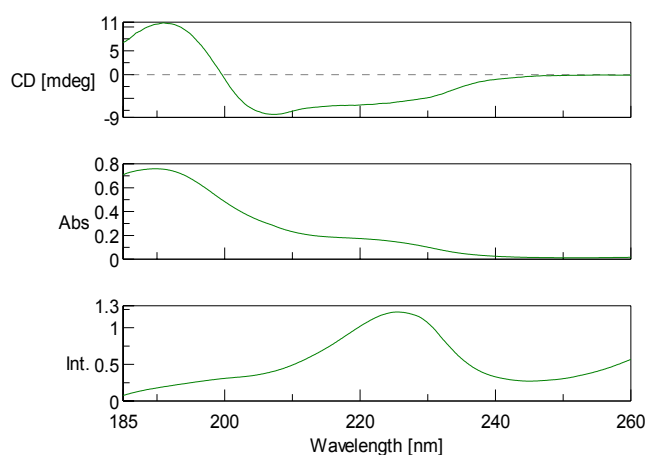


Fig. 4 Spectra of lysozyme

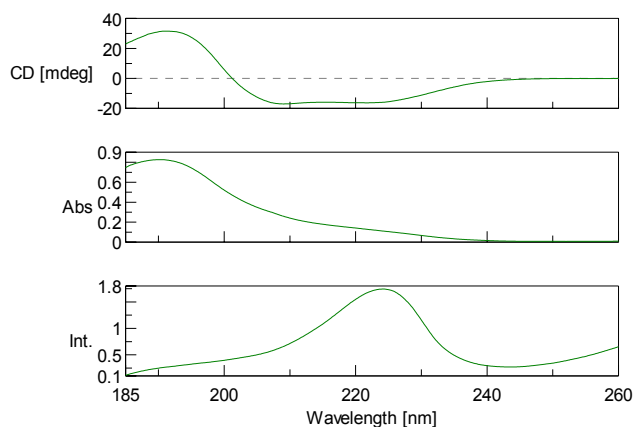


Fig. 5 Spectra of human serum albumin

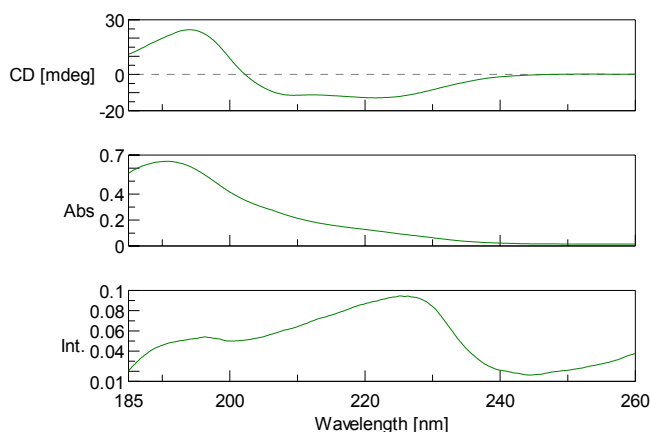


Fig. 6 Spectra of hemoglobin



Application Note

CD-0010

System configuration

J-815 CD Spectrometer
ASU-800 Autosampler
ASP-849 Syringe pump
JFLC-499 Peltier type flow cell for CD/Emission
FMO-427 Emission monochromator

Measurement condition

Light pathlength:	1 mm
Sample:	cytochrome c, lysozyme, human serum albumin, hemoglobin
Sample concentration:	0.1 mg/mL water solution
Wavelength range:	260 – 185 nm
Scan speed:	100 nm/min
Response:	2 sec.
Data acquisition interval:	0.1 nm
Band width:	1 nm
Accumulation:	4
Photometric mode:	CD, Abs, excitation spectrum
Emission wavelength:	350 nm, (sensitivity 850 V)
Temperature:	Room temperature