

High throughput CD Spectral Measurement by J-1500-PAL System. -Application to biomedicines, evaluation of pH dependency of human serum albumin structure-

Introduction

R&D of biomedicines utilizing active ingredient derived from protein is increasing day by day. However, such biomedicines are more sensitive against environmental change such as temperature, pH, and salt concentration than those of ordinary pharmaceuticals produced from low molecular compounds. And such sensitivity will be the possible cause of deactivation of biomedicines in production process.

The protein structure and its activity are related closely, while CD measurement can easily provide the information regarding the change of protein structure in small amount of sample. Therefore, the CD measurement is widely used in the quality control of biomedicines including proteins.

For such pharmaceutical lab, a fully automated high throughput CD spectral measurement system has been developed by JASCO in order to meet the demand for a great number of sample analyses in the short period. This system consists of model J-1500 CD spectrometer and a liquid handler, CTC PAL enabling the automation of sample pretreatment, injection and washing.

In this report, this automated system has been applied to the evaluation of pH dependency of human serum albumin (HAS) structure.

Keywords: Biomedicines, Quality control, Automated measurement/High throughput screening

System configuration

	Model	Description	P/N	Remarks
Main unit	J-1500-450	CD Spectrometer	7000-J006A	
Accessory	FLM-525	N ₂ gas flow meter	7069-J025A	
	PTC-517	Peltier Thermostatted Rectangular Cell Holder	7069-J017A	
	MCB-100	Mini water circulation bath	6970-J010A	For water-cooling of peltier
		Rectangular quartz cell, 10 mm path		For CD
	CTC-PAL	Liquid handler		

1) N₂ gas cylinder and regulator will be required additionally.

2) The 450W Xe source is recommended for offering higher S/N in vacuum UV region. A relevant water-circulator for cooling xenon lamp will be additionally required.

3) Please contact your local JASCO for the detailed configuration of CTC-PALPAL

Measurement parameters

Measurement program: [Spectra Measurement]

Reagent 1: 0.05 mg/mL human serum albumin (HAS) aqueous solution

Reagent 2: pH adjuster (pH1.3 - pH12.7, adjusted by diluted sulfuric acid and diluted sodium hydroxide aqueous solution)

Reagent 1 and reagent 2 were mixed into the ratio 1:4 and, the mixed reagent was injected into 10 mm rectangular cell placed in sample compartment of J-1500. All the sampling procedure such as mixing of reagents, CD spectral measurement, washing of cell and drying of cell have been pre-programmed so that a fully automated and unattended measurement can be carried out.

Data acquisition interval:	0.5 nm	Response:	1 sec
Band width:	1 nm	Scan speed:	100 nm/min
Optical path length:	10 mm	Accumulation:	2 times
Concentration:	0.01 mg/mL	Amount of HAS used for measurement:	30 µg

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Fig. 1 J-1500-PAL High Throughput system

Results

Fig. 2 shows the CD spectra of human serum albumin in each pH. As you can see, the CD spectra of HAS changed according to structural change by pH. Fig. 3 shows the change of intensity of CD peak at 222 nm (alpha-helix structure) against pH change. It indicates that in the pH range from 5 to 10, alpha helix rich structure is maintained. However, in the acidic conditions (below pH5) and basic conditions (over pH10), the CD intensity was decreased and, it suggests that the denaturation of HAS happened.

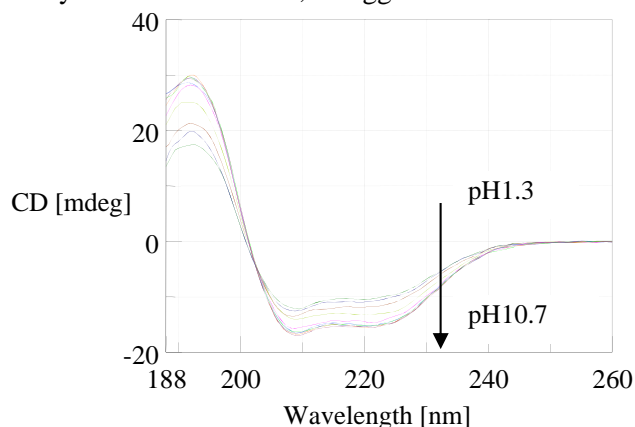


Fig. 2 CD spectra of HSA
(pH: 1.3, 2.2, 3.1, 4.1, 5.4, 6.7, 7.5, 8.4, 9.3, 10.7)

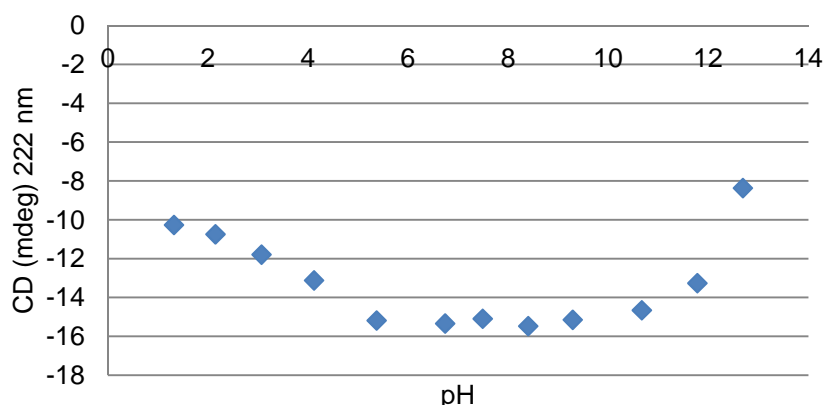


Fig. 3 pH dependency of CD intensity at 222 nm
(pH: 1.3, 2.2, 3.1, 4.1, 5.4, 6.7, 7.5, 8.4, 9.3, 10.7, 11.8, 12.7)

Conclusion

As demonstrated, the CD measurement can be the very effective procedure for quality control of biomedicines. The JASCO J-1500-PAL system will help the pharmaceutical labs who have a great number of samples to be measured.

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