

## Quality evaluation of biopharmaceuticals using circular dichroism spectrometer

### Introduction

Biopharmaceuticals have high specificity to diseased parts and have few side effects on the effect, and in recent years their market size is expanding more and more. On the other hand, compared with conventional low molecular weight medicines, its structure apt to change under the influence of temperature, solvent, pH, etc., and its function may be lost due to changes in manufacturing process etc. Therefore, it is very important to evaluate its stability and confirm its equivalence in the development and quality control of biopharmaceuticals.

Since the CD spectrum reflects the structure of proteins and nucleic acids and changes its shape, it is widely used for structural analysis and stability evaluation of these biopolymers. In recent years, using these features, the CD measurement method is used for development of biopharmaceuticals and comparison of equivalence between original drugs and biosimilars.

Here we introduce [Spectrum QC Test] program developed to apply the CD measurement method to confirm the equivalence of biopharmaceuticals. By using the statistical method, the [Spectrum QC Test] program can quantitatively determine whether the change in the CD spectrum is due to the noise of the equipment or the structural change of the protein. In this report, the structural change caused by thermal denaturation of human serum albumin was evaluated.

### Keywords

Biopharmaceuticals, quality control, biochemistry, circular dichroism measurement

### [Spectrum QC Test] program

- Quantitative evaluation is possible by judgment using statistical method (z test)
- Correlation coefficient, Euclidean distance, Manhattan distance are installed as comparison algorithm
- 21 CFR Part 11 compliant

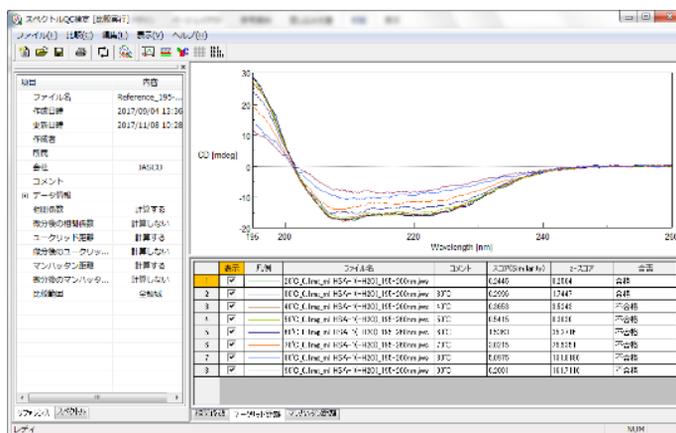


Fig. 1 [Spectrum QC Test] program

## Measurement

First, 10 CD spectra (1 mm optical path length, 0.1 mg/mL of concentration) of human serum albumin aqueous solution at 20°C were measured in order to obtain the average and deviation of reference spectrum group. Next, under the same condition as reference spectra, the CD spectra of human serum albumin aqueous solution were measured every 10°C from 20°C to 90°C, and the equality of CD spectra and reference spectral group at each temperature was judged by [Spectrum QC test] program.

### <Measurement Condition>

Scan speed:	100 nm/min	Response:	1 sec
Data acquisition interval:	0.1 nm	Spectral bandwidth:	1 nm
Accumulation:	1 time		

## Results

Figure 2-a shows the measurement result of 10 reference spectra measured at 20°C, and figure 2-b shows the change in CD spectra of human serum albumin aqueous solution with temperature rise. As the temperature rises, the intensity of CD spectrum decreases and the negative peak at 222 nm derived from the  $\alpha$ -helix structure becomes unclear.

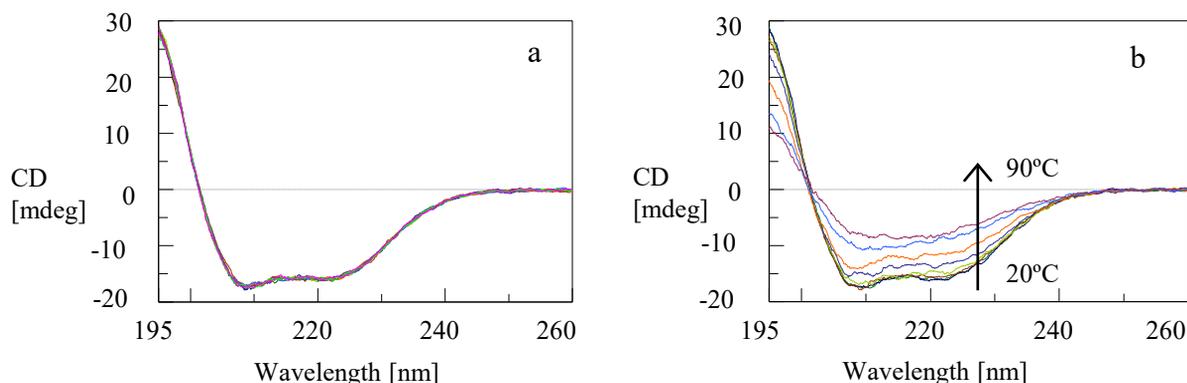


Fig. 2 CD spectra of human serum albumin aqueous solution  
 a: Reference spectra (20°C)  
 b: Changes in CD spectrum at 20°C to 90°C

## Analysis

By using the [Spectrum QC test] program, the similarity of the CD spectrum at each temperature was calculated from the Euclidean distance to the reference data group of human serum albumin aqueous solution at 20°C, and the equivalence to the reference spectrum group was judged by z test. From these results, it was found that the CD spectra of 20°C and 30°C measured under the same conditions as the reference spectrum group were judged to have the same spectral shape with respect to the reference spectral group, whereas at the temperature of 40°C or higher The CD spectrum was not judged to be equivalent.

In this way, by using the [Spectrum QC test] program, it is possible to quantitatively judge the equivalence of spectra in a statistical method, and to objectively judge structural changes in proteins.

Table 1 Results of Spectrum QC test

Temperature	Score( similarity)	Z score	Pass/Fail
20°C	0.2445	0.2504	Pass
30°C	0.2996	1.7447	Pass
40°C	0.3653	3.5243	Fail
50°C	0.5415	8.3030	Fail
60°C	1.5363	35.2716	Fail
70°C	3.0215	75.5351	Fail
80°C	5.0975	131.8180	Fail
90°C	6.2001	161.7110	Fail

### <Condition of spectrum comparison>

Algorithm: Euclidean distance  
 Comparison range: 195-260 nm  
 Z score criteria: 2 or less