Circular Dichroism
HTCD Plus

High-Throughput Circular Dichroism Measurement System

JASCO

For scientists in pharmaceutical, process- and biotechnology, and food chemistry labs who need reproducible, automated measurements and reliable structural analysis software, the High-Throughput Circular Dichroism (HTCD) system eliminates human error and bias in measurement acquisition and biomolecular characterization.

Availability of HTCD Plus

Circular Dichroism (CD) is well-known as the assessment method for monitoring the high-order structure of protein without complicated operation. In addition, it can also predict the component ratio of secondary structure in protein.

These capabilities makes CD well suited in the assessment of the following products:

R & D

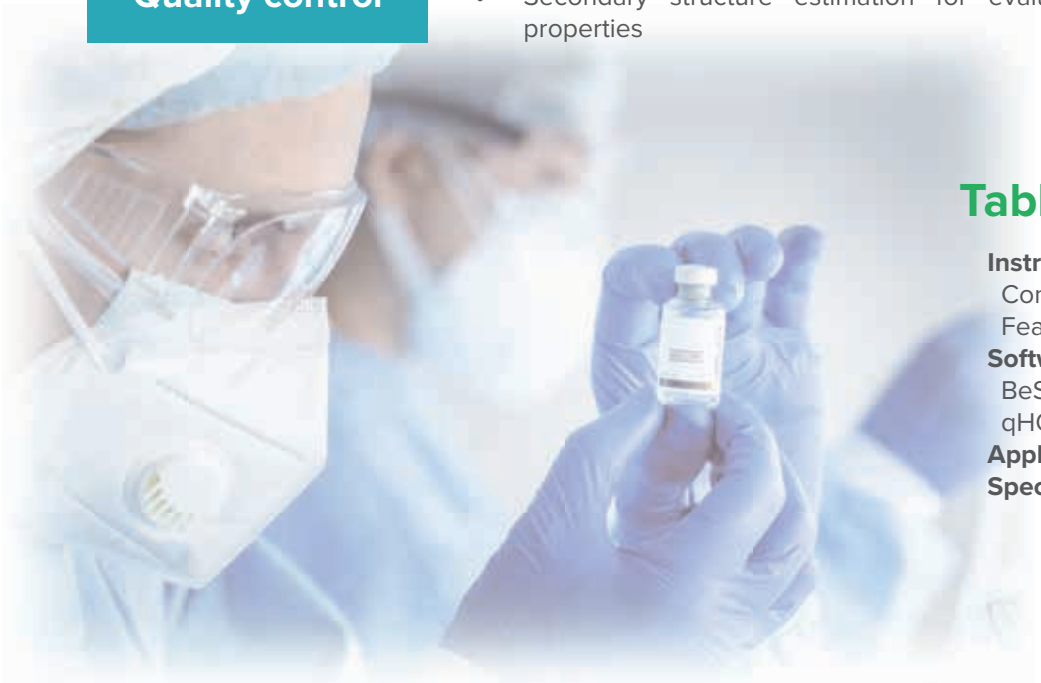
- Secondary/tertiary structure comparison between an innovator and a biosimilar
- Storage condition assessment

Manufacturing

- Monitoring secondary/tertiary structure changes in manufacturing processes

Quality control

- Secondary structure estimation for evaluating physical/chemical properties



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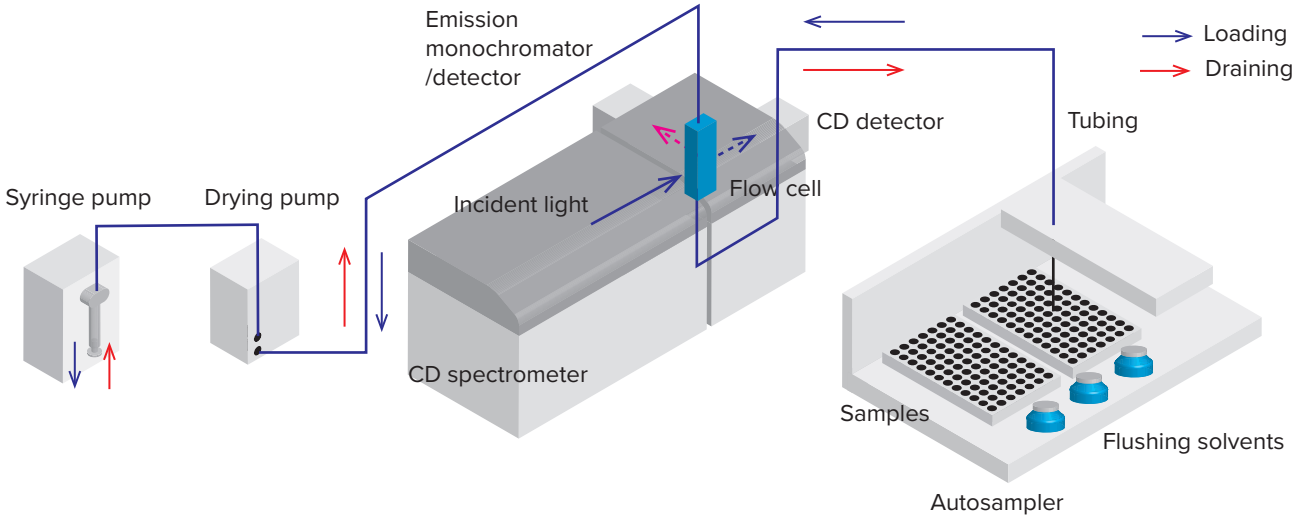
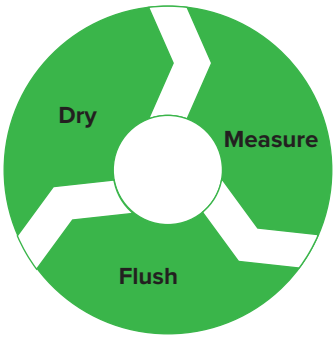
Automated Protein Screening

The evaluation of secondary and tertiary structure is important in quality control of protein and peptide based biologics. Circular dichroism (CD) is a sensitive probe of structure making it ideal for pharmaceutical stability and processing studies, where even slight changes to the molecule or its environment can induce structural changes, altering its function. While CD measurements are known to be quick and easy to perform, the high-throughput system dramatically increases the amount of data obtained with automated measurements using two 96-well microplates or 120 vials.



HTCD Plus overview

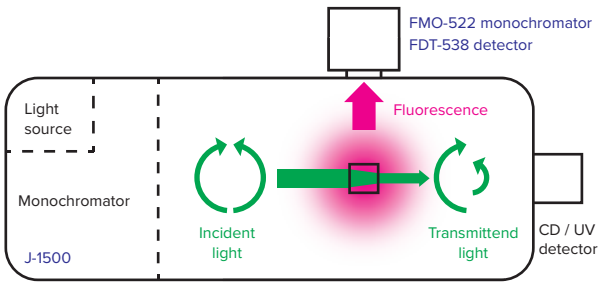
The HTCD allows automated scanning measurements using pre-programmed parameters. The autosampler can be set to maintain the microplate rack or vial rack at a constant temperature to prevent sample denaturation or evaporation. A system case with interlock prevents motion of the syringe arm while open. Pre-programmed flushing methods for protein or DNA/RNA samples to eliminate sample carry-over are included. Additionally, users can make customized flushing methods with up to three flushing solvents. The system allows samples to be recovered following measurement.



Measurement cycle

Simultaneous CD/fluorescence measurement

While CD spectroscopy provides information about secondary structure of proteins, fluorescence spectroscopy provides the information about tertiary structure. HTCD Plus can simultaneously measure CD and fluorescence with the use of the FMO-522 emission monochromator and FDT-538 detector.



Simultaneous CD/Fluorescence measurement

System configuration

JFLC-515 | Peltier Thermostated Flow Cell Holder

Accurate temperature controlled CD measurement accessory with a temperature range of 5 to 95 °C.

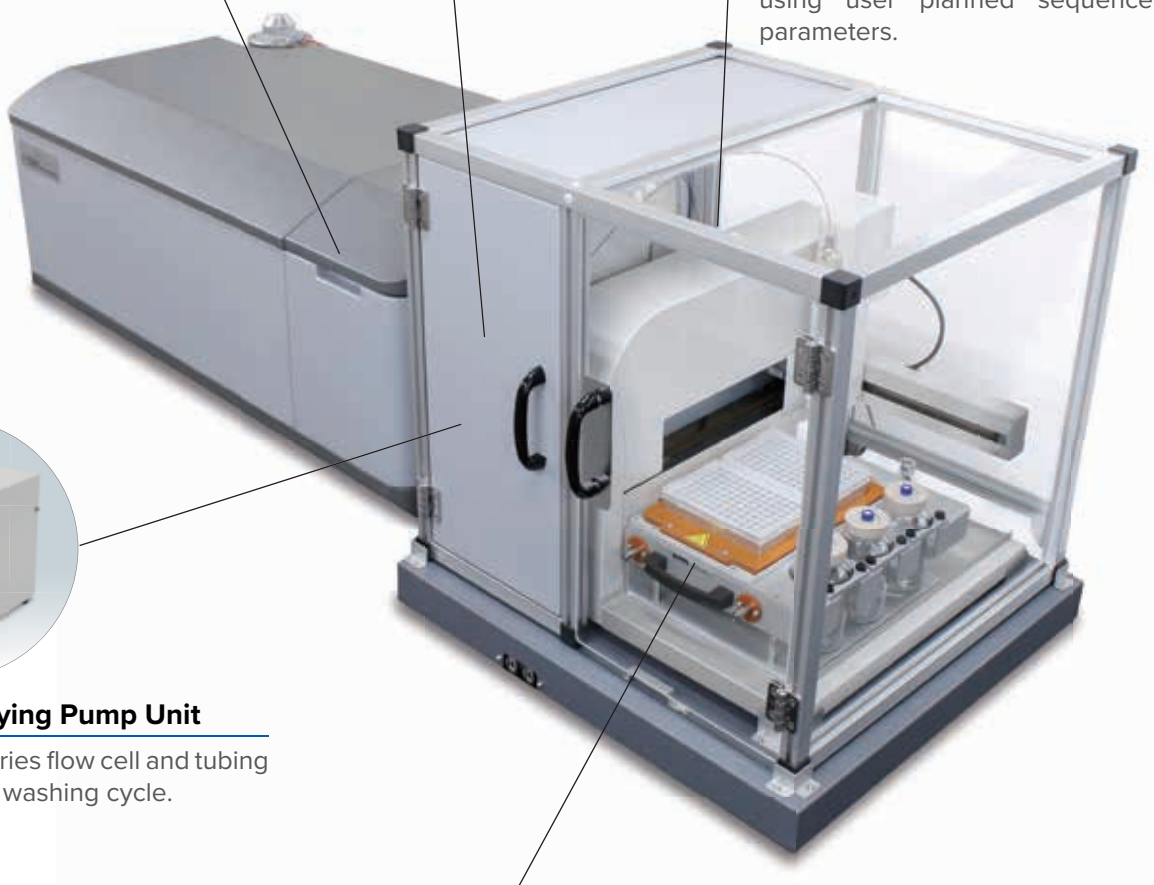
Flow Cell | Standard 1 mm pathlength cell: 25 µL
*0.2, 0.5 and 2 mm pathlength cells are options.

ASP-849 | Syringe Pump

High-accuracy pump for transferring as low as precise amount of sample to the flow cell.

ASU-800CD | Autosampler

Performs automated measurements using user planned sequence and parameters.



ADU-835 | Drying Pump Unit

Completely dries flow cell and tubing following the washing cycle.

SRA-841 | Microplate rack

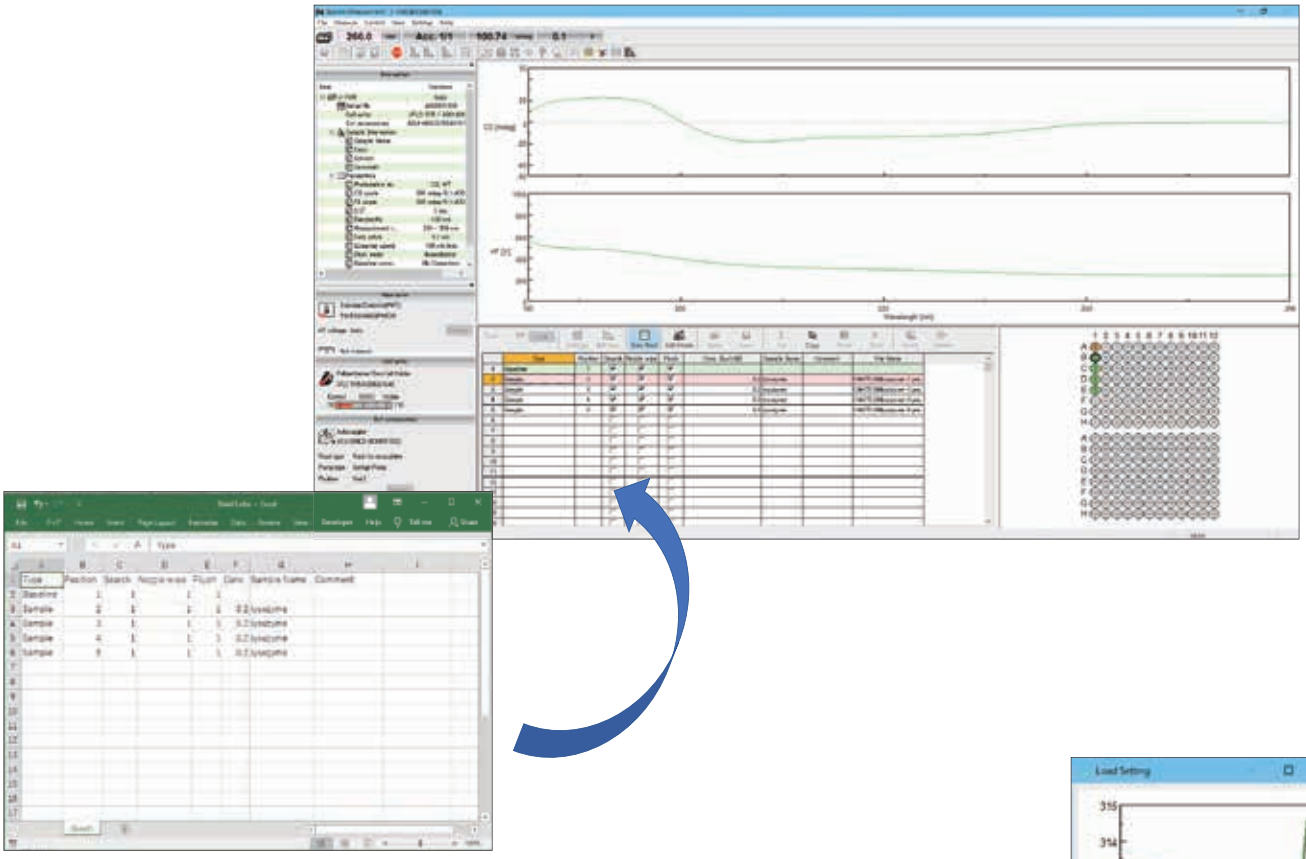
Holds up to two 96-well microplates and three flushing solvents (approx. 170 mL).

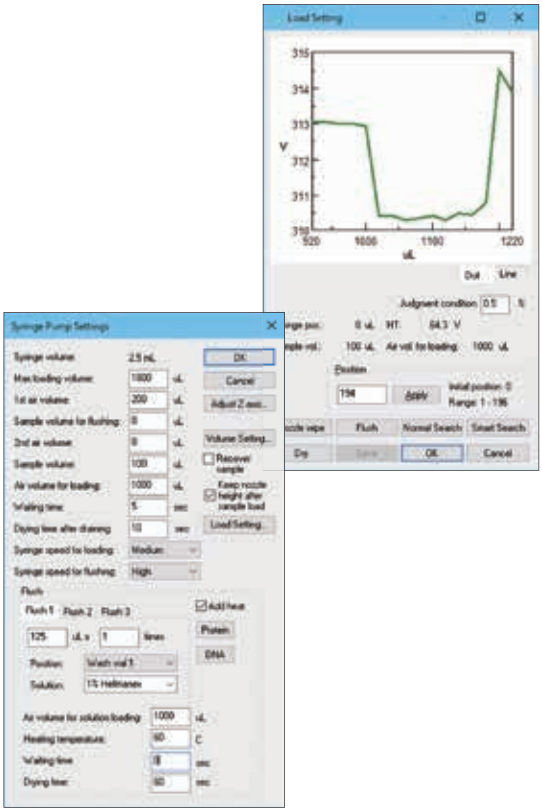
SRA-842 | Sample vial rack

Holds up to 120 sample vials and three flushing solvents (approx. 170 mL).

Advanced Features

- Fully automated measurement of up to 192 samples (two 96-well microplates), or 120 sample vials
- Pre-registered flush method for protein or DNA/RNA samples can be selected to eliminate sample carry-over
- Can be retrofitted onto an existing J-1500
- Flow monitor function to optimize the sample flow and loading





Sequence Program

A measurement sequence can be created in Spectra Manager™ or imported using a spreadsheet.

Air Volume Auto Optimization

The air volume for loading sample to the flow cell is determined automatically (or manually) by photometric measurements, which allows sample volume optimization. This function helps to reduce the dead-volume.

Pre-programmed Flushing Function

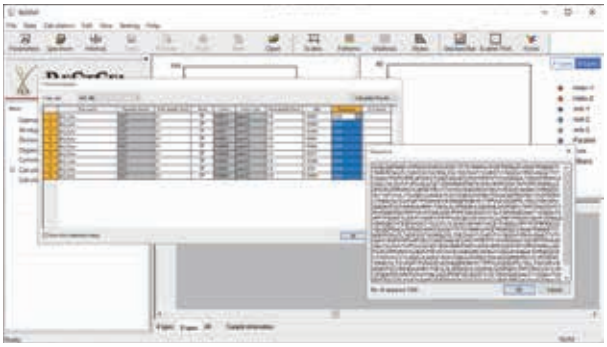
Flushing methods for protein and DNA/RNA samples are pre-programmed to eliminate sample carry-over. Flushing methods can be customized with up to three flushing solvents.

Validation and data confidence

Count on the accuracy and repeatability of your data. An integrated validation mode provides users with up to nine instrument performance and calibration tests. Each J-1000 system includes a built-in Hg lamp wavelength calibration source. JASCO also offers the first traceable scale calibration substance (d-10-ammonium camphorsulfonate) for photometric accuracy and repeatability tests.

Spectra Manager BeStSel program (Option)

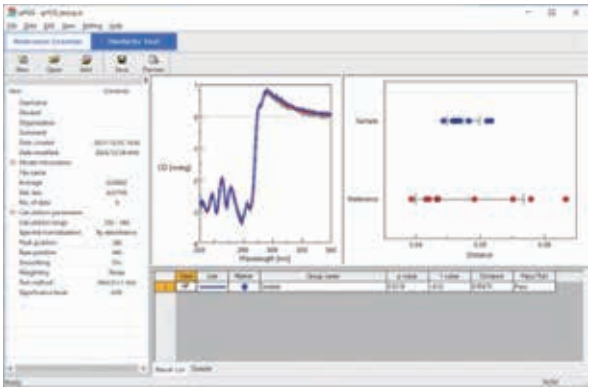
Recently, Micsonai et al. developed the BeStSel algorithm that can accurately estimate the secondary structure composition from the CD spectrum by taking into account the parallel-antiparallel orientation of the β-strands and the twist of the antiparallel β-sheets. An offline BeStSel software in Spectra Manager Ver. 2.5 (Spectra Manager BeStSel) was jointly developed, and enables seamless analysis in an offline capacity.



qHOS program (Option)

qHOS statistically determines the difference between spectra, taking into consideration various error factors. Features of the qHOS program include:

- Statistical similarity evaluation
- Robust evaluation using a noise weighting method
- Student, Welch, and TOST *t*-test implementation
- Auto concentration correction
- Can be used with different measurement types: FTIR, Fluorescence, UV-Vis etc.
- Regulatory compliance with Spectra Manager™ 2.5 CFR



Applications

Spectral identity studies of therapeutic antibodies

Here we outline the results of HOS similarity assessment of RIABNI™ (a biosimilar to MabThera®/Rituxan®, the innovator of Rituximab), and Herceptin® (the innovator of Trastuzumab), using the HTCD Plus and the qHOS program.

Figure 1 shows the similarity assessment result of the tertiary structures of MabThera® and Herceptin®. The system clearly distinguishes differences in the tertiary structure of different antibody drugs giving a similarity fail result.

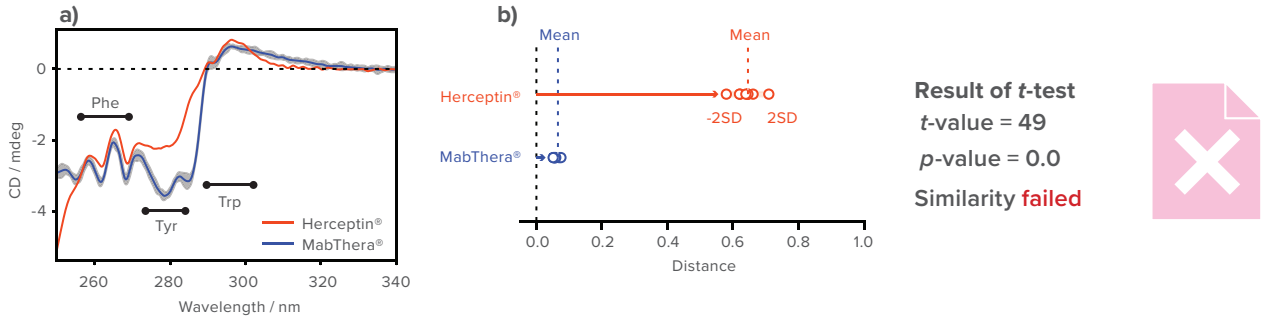


Figure 1. Results of HOS similarity assessment for different antibody drugs

a) Mean spectra of MabThera® (blue), Herceptin® (red), and standard deviation for MabThera® (gray).

b) Distance test results for MabThera® (blue) and Herceptin® (red).

Figures 2 and 3 show the similarity assessment results of the secondary and tertiary structures of MabThera® and RIABNI™, respectively. These results objectively confirmed that RIABNI™ has the same tertiary and secondary structures as MabThera®.

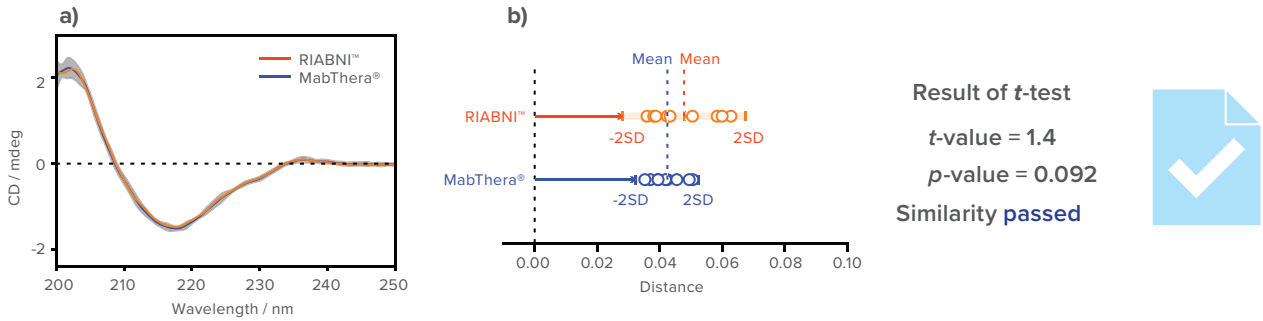


Figure 2. Results of HOS similarity assessment for Rituximab innovator (blue) and biosimilar (red) by far-UV/CD

a) Mean spectra of MabThera® (blue), RIABNI™ (red), standard deviation for MabThera® (gray).

b) Distance test results for MabThera® (blue) and RIABNI™ (red).

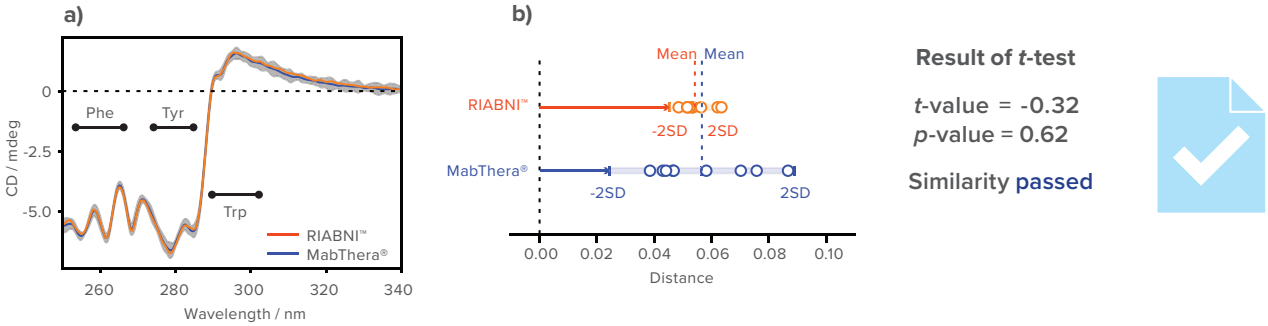


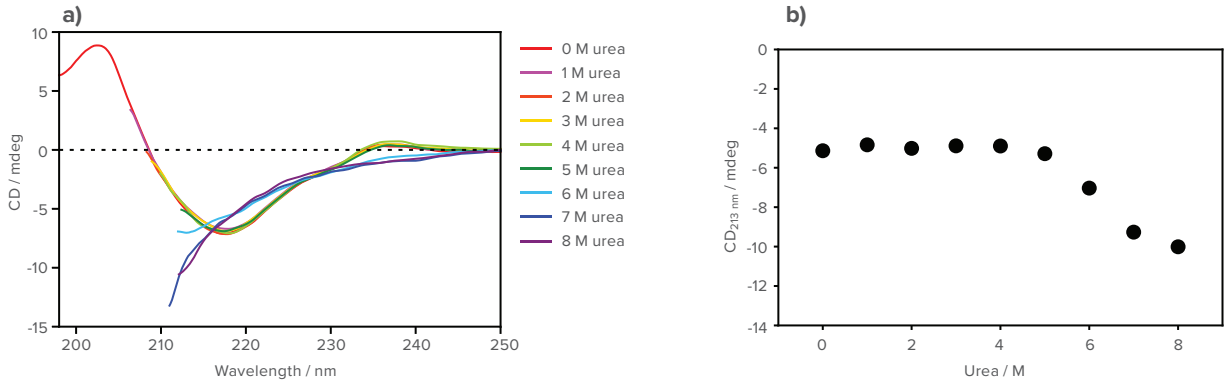
Figure 3. Results of HOS similarity assessment for Rituximab innovator (blue) and biosimilar (red) by near-UV/CD

a) Mean spectra of MabThera® (blue), RIABNI™ (red), standard deviation for MabThera® (gray).

b) Distance test results for MabThera® (blue) and RIABNI™ (red).

Stability assessment by CD/fluorescence spectroscopy

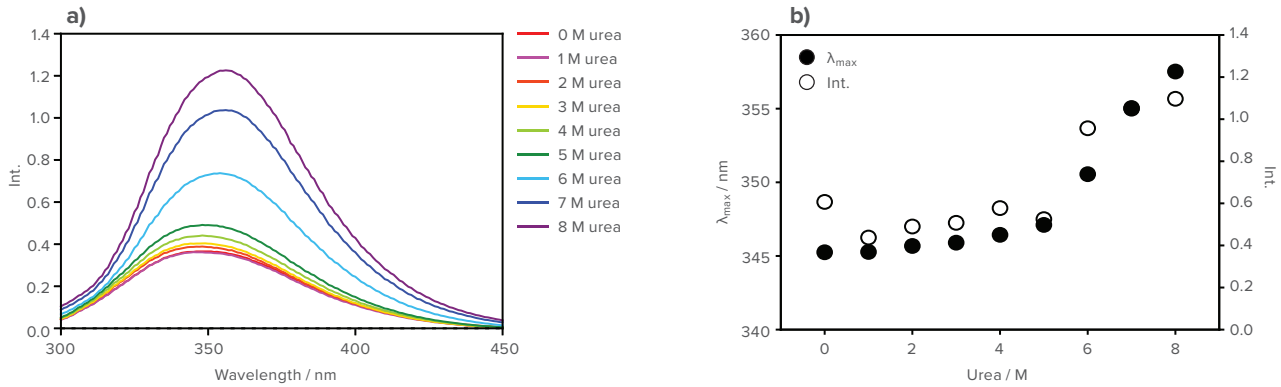
CD and fluorescence spectroscopies are well-known as methods for assessing the conformation of proteins in solution. Generally, CD and fluorescence spectra are obtained using different instruments; however, combining HTCD Plus with the fluorescence measurement unit enables both spectra to be obtained efficiently. This system is effective at developing therapeutic antibodies which require rapid screening to find poteintial candidates. Here the stability assessment of a therapeutic antibody (rituximab) against different concentrations of urea is outlined.



Results of stability assessment for therapeutic antibody by CD spectroscopy

a) CD spectra of a therapeutic antibody (rituximab) with different concentrations of urea,

b) CD signal at 213 nm as a function of urea concentration



Results of stability assessment for therapeutic antibody by fluorescence spectroscopy

a) Fluorescence spectra of a therapeutic antibody (rituximab) with different concentrations of urea

b) Fluorescence signal and wavelength with maximum fluorescence as a function of urea concentration

Batch processing of secondary structure estimation

The secondary structure of protein varies due to changes in the surrounding environment, such as pH and salt concentration. In order to know the secondary structure composition of protein under various conditions, many measurements can be performed using HTCD Plus, and the secondary structure estimation results are obtained from for all spectra at once.

BeStSel CFR software can perform the batch processing of many spectra data for secondary structure estimation, and can provide all secondary structure estimation results visually.

pH and salt-induced denaturation study of VHH antibody

Here we give an overview of a stability evaluation of antibodies (VHH model) by comparing the CD spectra of native and denatured antibodies using statistical analyses. Figure 1 shows the CD spectra of VHH solutions with different pH values, and Figure 2 shows the evaluation results for VHH structural changes correlated with pH and NaCl concentration. The qHOS program quantifies the similarity of CD spectra using a statistical method and quantitatively evaluates CD spectral changes associated with structural changes in proteins.

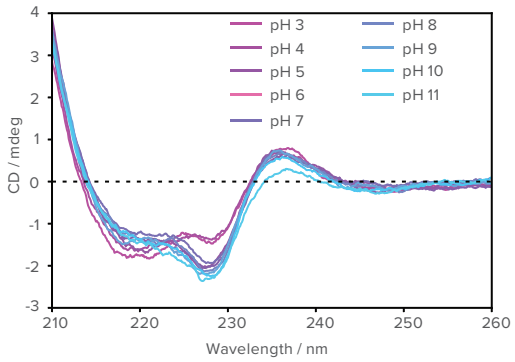


Figure 1. CD Spectra of VHH antibody

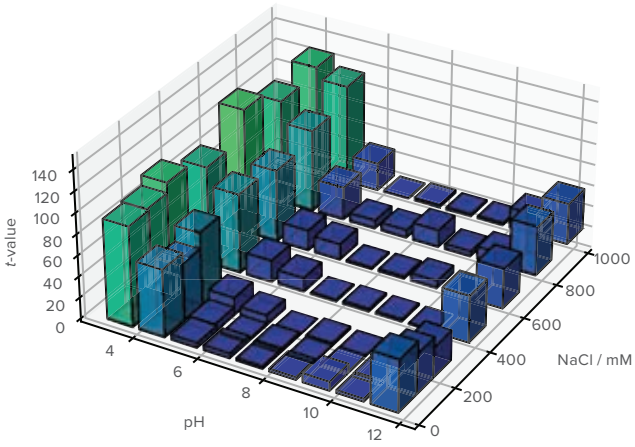


Figure 2. Relation between pH and NaCl concentration of VHH antibody

Figure 3 shows a plot of the *t*-value obtained by HTCD and T_m obtained by denaturation temperature measurement. The high correlation between the *t*-value and the denaturation temperature suggests that a spectral difference test is a very useful primary screening method before performing a thermal denaturation analysis, which generally requires a great deal of time.

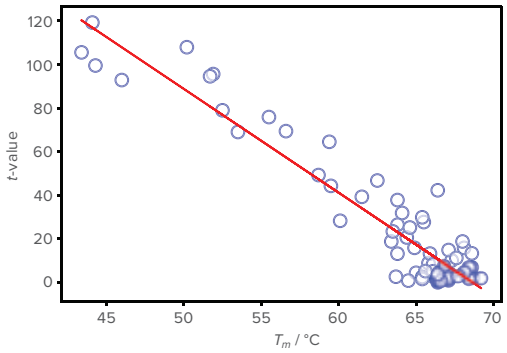


Figure 3. Relation between denaturation temperature and *t*-value

MPTC-513 Automatic 6-position Peltier turret cell changer allows temperature control using the temperature control program, and allows the measurement of the change in CD values of a maximum of six samples.

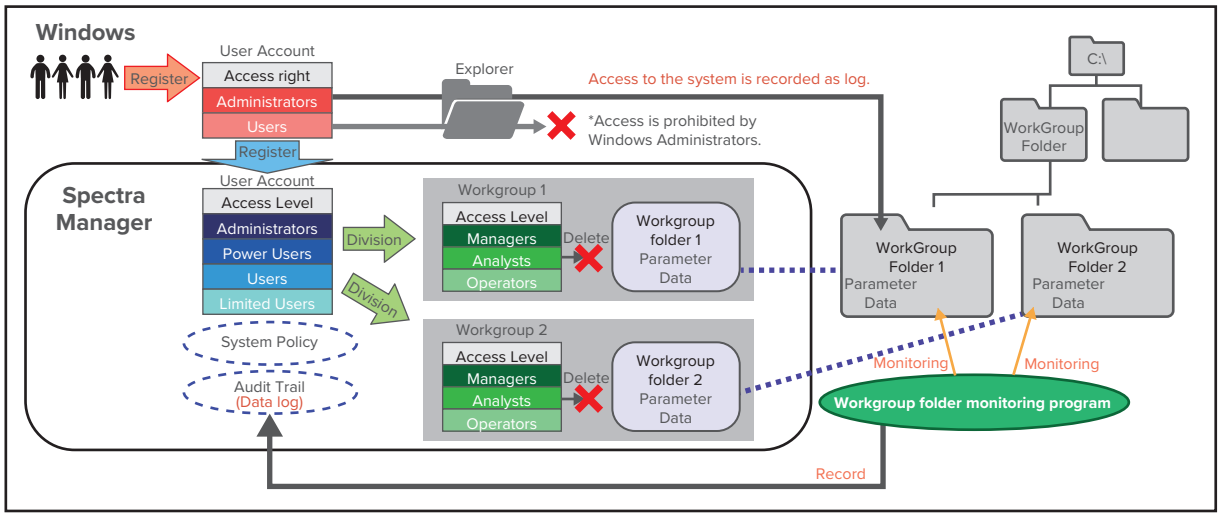
In the screening analysis, 10.5 hours are required to measure CD spectra for about 84 samples with different pHs values and salt concentrations. The *t*-value is then calculated using the qHOS program. It then takes 2.5 hours to measure T_m values for about 12 selected samples (2 sets of six simultaneous measurements) using a turret-type cell changer and the temperature interval measurement program to vary the temperature from 20 to 82 °C. The HTCD Plus allows the entire set of samples to be evaluated in only about 12 hours.

VHH antibody was kindly provided by RePHAGEN https://rephagen.com/

Special thanks for collaboration; Prof. Kouhei Tsumoto, School of Engineering and Institute of Medical Science, The University of Tokyo

Regulatory Compliance with Spectra Manager™ 2.5 CFR

JASCO's Spectra Manager™ 2.5 CFR is designed and developed under ALCOA+ principles and is a total solution platform to create accurate and complete data.



Overview

Solution for data integrity

Enduring Electronic Record

Based on prohibiting the function to delete electronic records and to overwrite saves, while maintaining the function to backup and restore data, electronic records can be saved properly and searched accurately during the data lifecycle.

User Management

Two security categories [Access Level] and [Work Group], allow flexible and independent authorization of users and projects for different applications, instruments and measurement programs.

Audit Trail

Data is categorized and recorded as 3 different records (system log, application log and data log). Each log can be filtered and displayed by recorded date, user name, etc., which can then be exported for audit trail review.

User Account Security

Functions to prevent duplicate accounts, protect passwords and prevent unauthorized access, administrative authorizations, such as system access and electric signatures, etc., can be managed strictly.

Computerized System Validation

JASCOS's Spectra Manager™ 2.5 CFR is designed, developed and manufactured properly under quality control system adapted ISO 9001, and adapted CSV standard.

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