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.

The above features enable to perform the assessment of the following products.

Therapeutic antibodies

Biosimilar

Antibody-drug conjugate (ADC)

Therapeutic peptides/proteins

Therapeutic oligonucleotides/mRNAs

R & D

- The secondary/tertiary structure comparison between innovator and biosimilar
- Storage condition assessment

Manufacturing

- Monitoring of the secondary/tertiary structure changes in manufacturing process

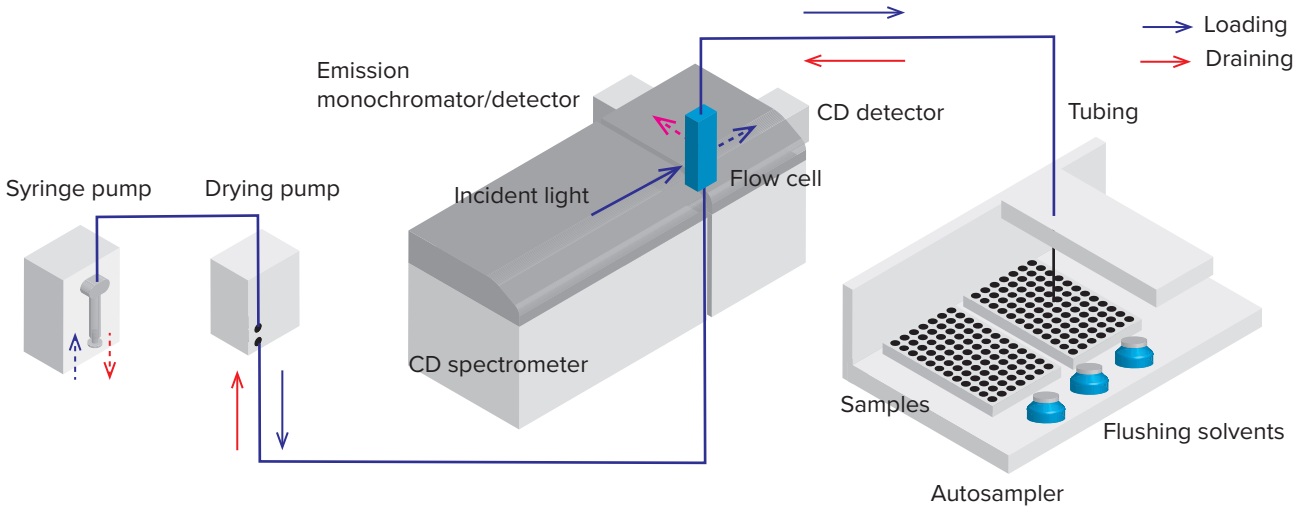
Quality control

- Secondary structure estimation for evaluating the physical/chemical property

Table of contents

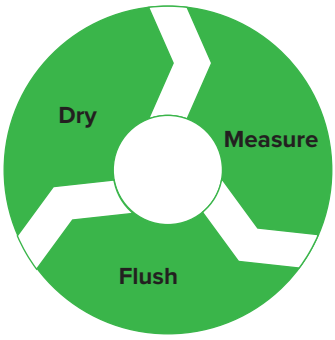
Automated Protein Screening

The evaluation of secondary and tertiary structure is important in quality control of protein and peptide based biologics. Circular dichroism (CD) is sensitive to a biomolecule's asymmetry and is 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 and 120 vials.



HTCD Plus overview

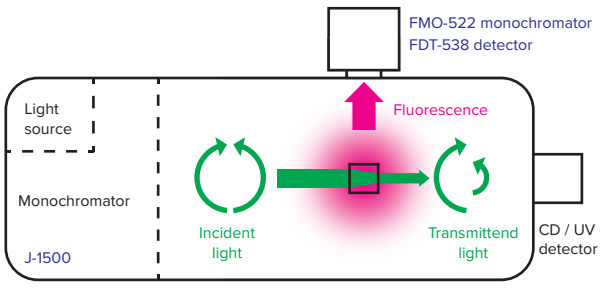
The HTCD allows automated scanning measurements using pre-programmed parameters. The autosampler can be set to maintain the microplate rack or vial rack at a constant temperature to prevent sample denaturation or evaporation, and a system case has the interlock to prevent from harming the operators. The flushing method is pre-programmed for protein or DNA/RNA samples to eliminate sample carry-over, and the method is also customizable with up to three flushing solvents. The system allows sample recovery after measurement, and batch data processing includes secondary structure and comparability analysis.



Measurement cycle

Simultaneous CD/fluorescence measurement

While CD spectroscopy can provide the information on the secondary structure of protein, fluorescence spectroscopy can provide the information on the tertiary structure. HTCD Plus has the capability for performing simultaneous CD/fluorescence measurement.



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.

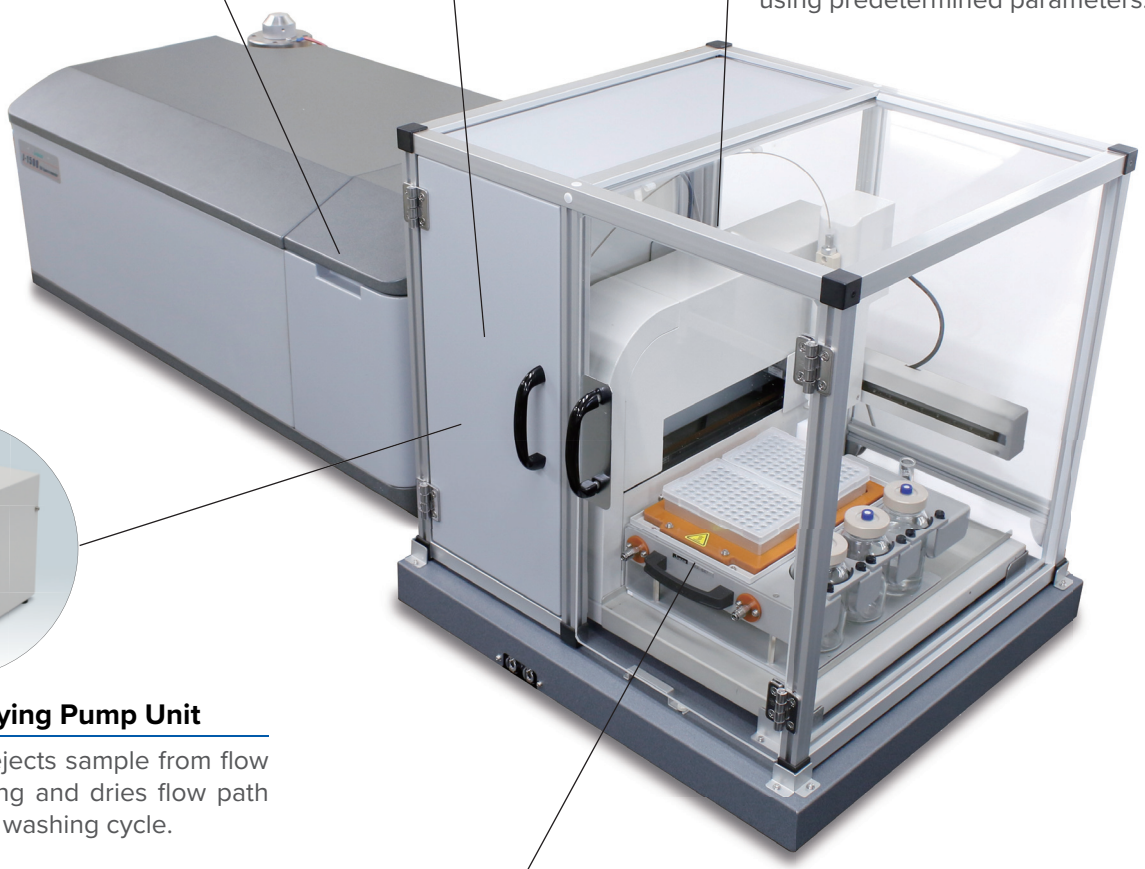
Flow Cell | Standard 1 mm pathlength cell: 25 µL
*0.2, 0.5 and 2 mm pathlength cells are options.

ASP-849 | Syringe Pump

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

ASU-800CD | Autosampler

Performs automated measurements using predetermined parameters.



ADU-835 | Drying Pump Unit

Completely ejects sample from flow cell and tubing and dries flow path following the washing cycle.

SRA-841 | Microplate rack

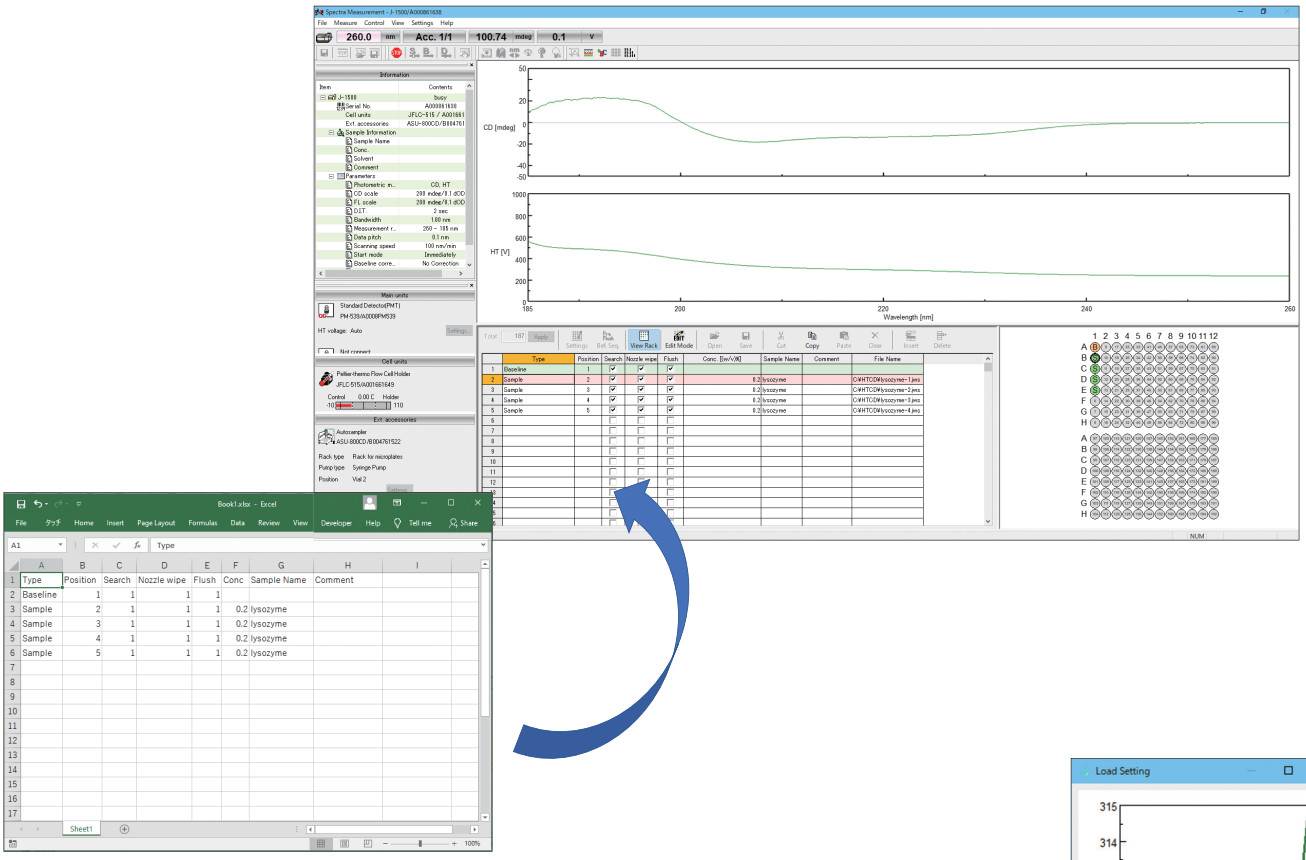
Holds up to two 96-well microplates and three flushing solvents (approx. 170 mL).

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
- Retrofit capability to J-1500 CD spectrometer
- Flow monitor function to optimize the sample flow condition



Sequence Program

A sequence program can be created on Spectra Manager™, and can be imported using spreadsheet.

Air Volume Auto Optimization

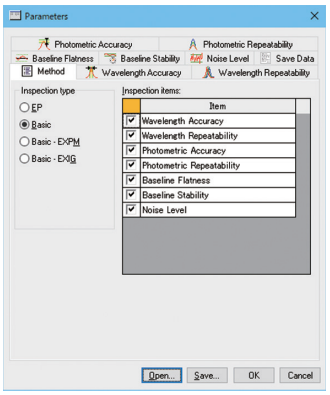
The air volume for loading sample to the flow cell is determined automatically (or manually) by photometric measurements, which allows sample volume optimization. This function helps to reduce the dead-volume.

Pre-programmed Flushing Function

The flushing method is pre-programmed for protein or DNA/RNA samples to eliminate sample carry-over, and the method is also customizable with up to three flushing solvents.

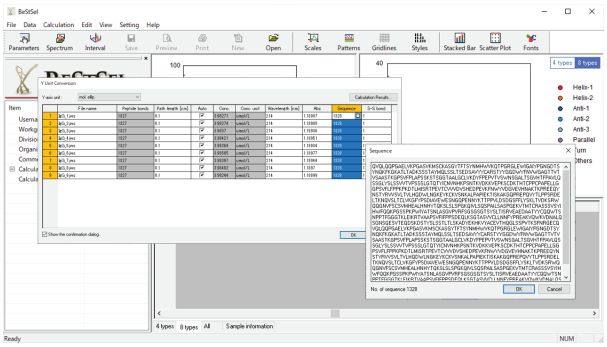
Validation and data confidence

Count on the accuracy and repeatability of your data. An integrated validation mode provides users with a list of 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.



BeStSel CFR program (Option)

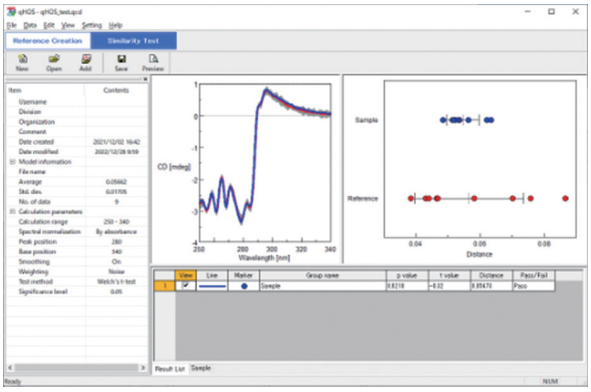
Recently, András Micsonai et al. developed the BeStSel algorithm that can accurately estimate the secondary structure composition from the CD spectrum by taking into account the parallel-antiparallel orientation of the β-strands and the twist of the antiparallel β-sheets. Spectra Manager™ 2.5 CFR BeStSel offers a control and analysis platform for CD spectrometers, which is compatible with GxP and satisfying Computerized System Validation (CSV), Electronic Records/Electronic Signatures (ER/ES), and Data Integrity (DI) for practice ALCOA+.



qHOS program (Option)

The qHOS can statistically determine the significant difference between spectra, considering various error factors and with the following features.

- Statistical similarity evaluation
- Robust evaluation using noise weighting method
- Student, Welch, TOST *t*-test implementation
- Auto concentration correction
- Orthogonal similarity assessment
- Regulatory compliance with Spectra Manager™ 2.5 CFR



Applications

Spectral identity studies of therapeutic antibodies

This section shows the results of an 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®. These results show that the system can clearly distinguish differences in the tertiary structure of different antibody drugs.

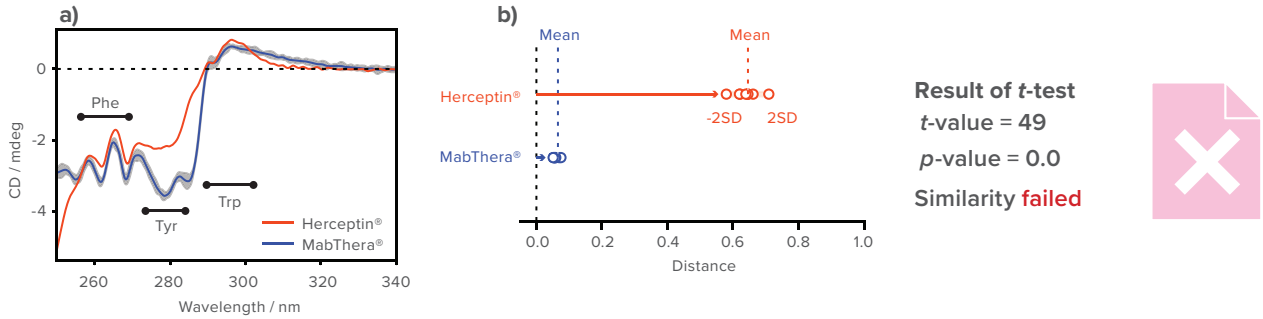


Figure 1. Results of HOS similarity assessment for different antibody drugs

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

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

Figure 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®.

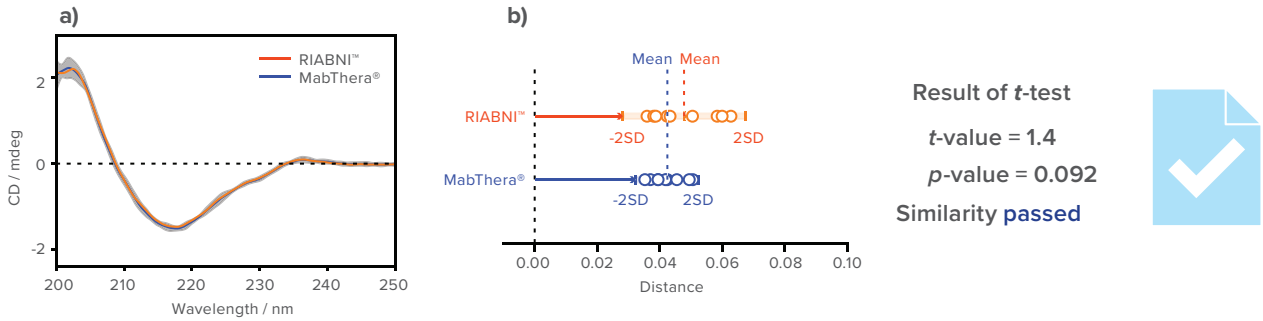


Figure 2. Results of HOS similarity assessment for Rituximab innovator (blue) and biosimilar (red) by far-UV/CD

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

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

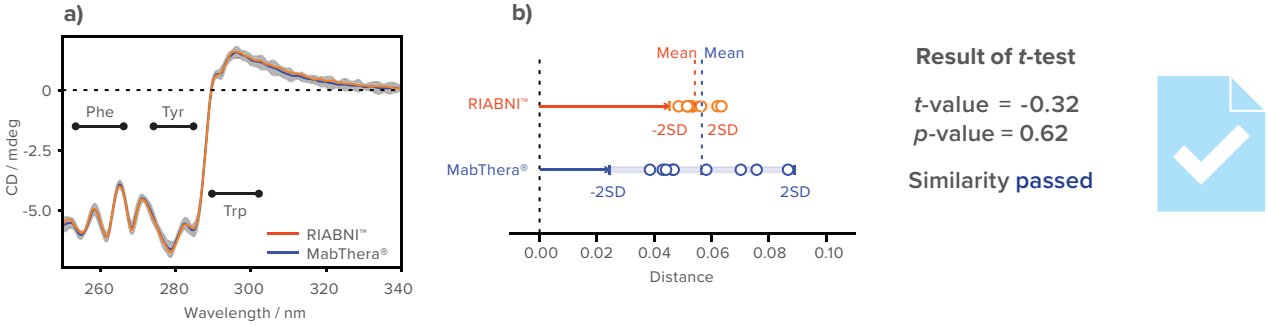


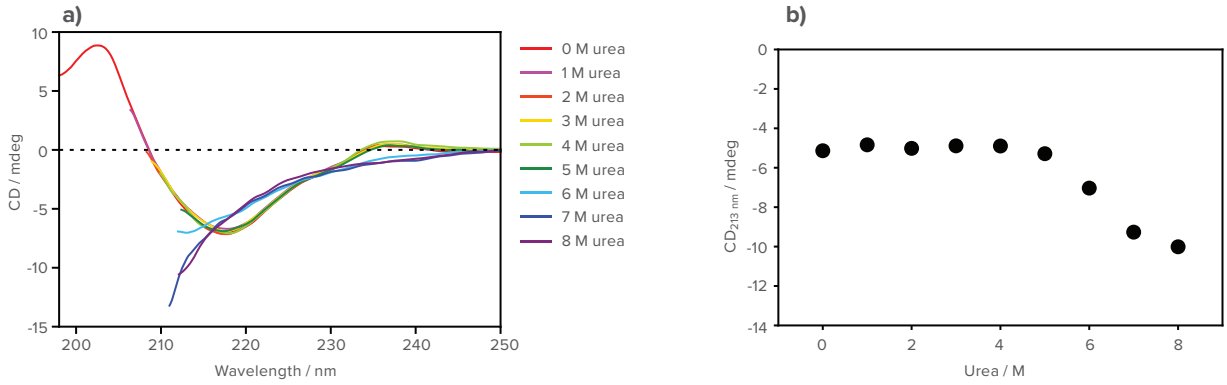
Figure 3. Results of HOS similarity assessment for Rituximab innovator (blue) and biosimilar (red) by near-UV/CD

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

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

Stability assessment by CD/fluorescence spectroscopy

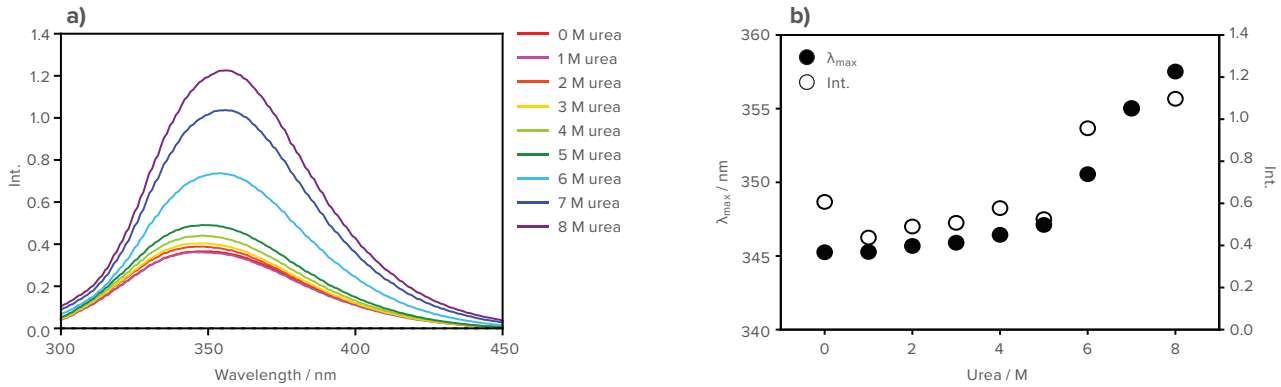
CD and fluorescence spectroscopies are well-known as the methods for assessing the conformation of protein in solution. Generally, CD and fluorescence spectra are obtained by the different instrument respectively. Combining HTCD Plus with the fluorescence measurement unit enables to obtain the both spectra effectively. This system is effective at the development of the therapeutic antibody which needs the screening for finding the candidates. This shows the stability assessment example of therapeutic antibody (rituximab) against the urea.



Results of stability assessment for therapeutic antibody by CD spectroscopy

a) CD spectra of therapeutic antibody with the different concentration urea

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



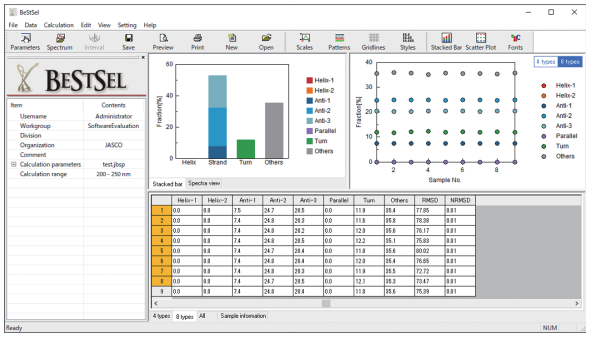
Results of stability assessment for therapeutic antibody by fluorescence spectroscopy

a) Fluorescence spectra of therapeutic antibody with the different concentration urea

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

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 should be performed by using HTCD Plus, and it is preferred that the secondary structure estimation results are obtained from many data automatically.
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

This shows the result of 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 for solutions with different pH values, and Figure 2 shows the evaluation results for VHH structural changes with the pH and NaCl concentration. qHOS program can quantify the similarity of CD spectra using a statistical method, and can quantitatively evaluate CD spectral changes associated with structural changes in proteins.

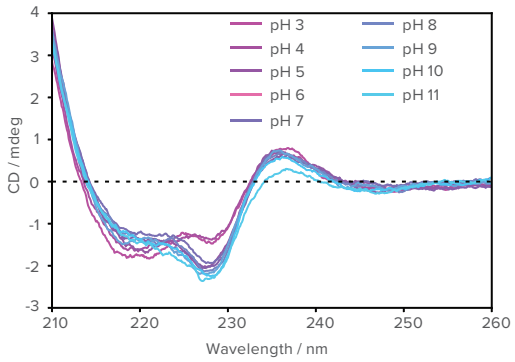


Figure 1. CD Spectra of VHH antibody

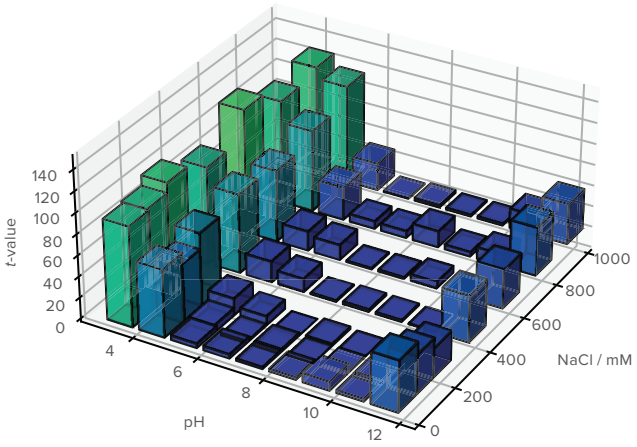


Figure 2. Relation between pH and NaCl concentration of VHH antibody

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.

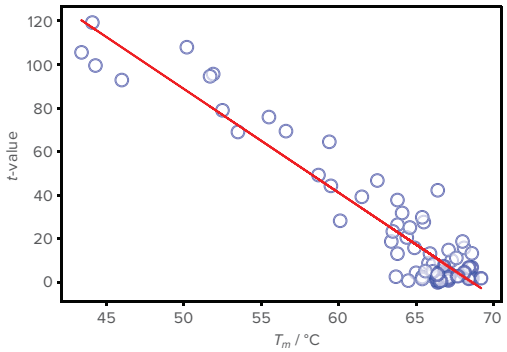


Figure 3. Relation between denaturation temperature and *t*-value

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 up to six samples.

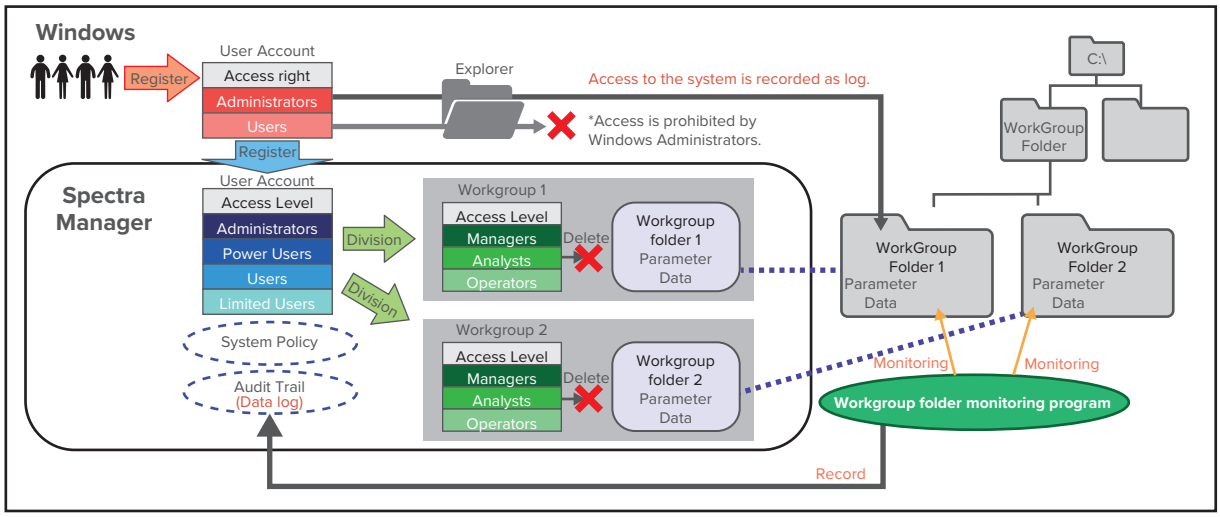
In the screening analysis, 10.5 hours are required to measure CD spectra for about 84 samples with different pHs values and salt concentrations. The *t*-value is then calculated using qHOS program. It then takes 140 minutes to measure T_m values for about 12 selected samples (2 sets of six simultaneous 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 Spectra Manager™ 2.5 CFR is designed and developed under ALCOA+ and is a total solution platform to create accurate and complete data.



Overview

Solution for data integrity

Enduring Electronic Record

Based on prohibiting function to delete electronic record and to overwrite save, and also functions for backup and restore data, electronic records can be saved properly and can be searched accurately during the data lifecycle.

User Management

Based on the dual security category ([Access Level] and [Work Group]), it is possible to manage different authorization processes in flexible and independent as total analysis systems, instrumentations and analytical applications.

Audit Trail

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

User Account Security

Based on functions to prevent account duplication or to protect password, and to prevent unauthorized access, administrative authorizations such as system access and electronic signature etc., can be managed strictly.

Computerized System Validation

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

JASCO CORPORATION
2967-5, Ishikawa-machi, Hachioji-shi, Tokyo 192-8537 Japan
Tel: +81-42-649-5177 Fax:+81-42-646-4515 Web: www.jasco.co.jp
Japan

JASCO INTERNATIONAL CO., LTD.
11-10, Myojin-cho 1-chome, Hachioji-shi, Tokyo 192-0046, Japan
Tel: +81-42-649-3247 Fax: +81-42-649-3518 Web: www.jascoint.co.jp/english/
Australia, Hong Kong, India, Indonesia, Korea, Malaysia, New Zealand, Pakistan,
Philippines, Russia and CIS countries, Singapore, Taiwan, Thailand, Vietnam

JASCO INCORPORATED
28600 Mary' s Court, Easton, Maryland 21601, U.S.A.
Tel: +1-410-822-1220 Fax: +1-410-822-7526 Web: www.jascoinc.com
Argentina, Bolivia, Brazil, Canada, Chile, Colombia, Costa Rica, Guatemala, Mexico,
Paraguay, Peru, Puerto Rico, United States of America, Uruguay, Venezuela

JASCO EUROPE S.R.L.
Via Luigi Cadorna 1, 23894 Cremella (LC), Italy
Tel: +39-039-9215811 Fax: +39-039-9215835 Web: www.jascoeurope.com
JASCO Deutschland www.jasco.de | **JASCO UK** www.jasco.co.uk | **JASCO France** www.jascofrance.fr
JASCO Benelux www.jasco.nl | **JASCO Spain** www.jasco-spain.com
Algeria, Austria, Belgium, Cyprus, Denmark, Egypt, Finland, France, Germany, Greece, Hungary,
Israel, Italy, Jordan, Kuwait, Luxembourg, Morocco, Netherlands, Norway, Poland, Portugal, Romania,
Saudi Arabia, South Africa, Spain, Sweden Switzerland, Tunisia, Turkey,
United Arab Emirates, United Kingdom, Yemen

JASCO CHINA (SHANGHAI) CO., LTD.
Room No.D, 10F, World Plaza, 855 Pudong South Road, Pudong New Area, Shanghai, China
Tel: +86-21-6888-7871 Fax: +86-21-6888-7879 Web: www.jasco-global.com
China

ISO-9001
ISO-14001
CERTIFIED

Products described herein are designed and manufactured by ISO-9001 and ISO-14001 certified JASCO Corporation

DE6603-2303-01