# Stability Evaluation of Trastuzumab and Rituximab Using Automated Circular Dichroism (CD) and Fluorescence Spectroscopy

## Introduction

Antibody therapeutics have been dramatically expanding their market over the past decades and have become major biotherapeutic proteins. Circular dichroism (CD) and fluorescence spectroscopies are both easy yet sensitive techniques for studying conformational changes in solution-state proteins. CD spectroscopy measures the difference in absorption of left- and right-handed circularly polarized light. In the far-UV region, CD signals are produced by optical absorption by the peptide backbone, and are therefore used to observe changes in protein secondary structure. Notably, CD spectroscopy is used to compare the higher-order structure (HOS) and homogeneity before and after a change in the manufacturing process as specified in ICH Q5E[1], and to compare the HOS of innovators and biosimilars as described in FDA[2], EMA[3] and other guidelines[4,5]. Intrinsic fluorescence spectroscopy is a complementary technique for studying protein structure. When proteins are exposed to UV light, fluorescence is emitted by their aromatic amino acid residues. The changes in emission intensity and wavelength are affected by the local environment of these residues. Conventionally, CD and fluorescence spectra are obtained using two different instruments. However, when using multiple instruments, the following challenges are raised: a) time and effort for physical transfer of a sample between instruments, and b) possible difference in sample conditions. These are particularly crucial for these two techniques, since they are often employed as screening methods for a large number of candidates. To address these challenges, we developed an automatic measurement system for CD and fluorescence spectra. The system is composed of the J-1500 CD spectrometer combined with the HTCD Plus automatic CD measurement unit, and a fluorescence monochromator. Using this system, we conducted a comprehensive stability evaluation for trastuzumab (Herceptin®) and rituximab (RIABNI™) by changing the urea concentration and pH. We believe that this new system is especially useful for early-stage screening of therapeutic antibody candidates.

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## Experimental

### Materials

• Herceptin® 150 mg
(trastuzumab, Roche)

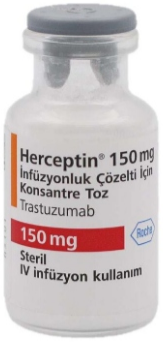


*Fig. 1 Image of Herceptin®*

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• RIABNI™ 10 mg/ mL
(rituximab, AMGEN)


*Fig. 2 Image of RIABNI™*

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# High-Throughput CD Measurement System

*Figure 3. Image of HTCD Plus system*

## System Features

- Automated sample loading, wavelength scanning and flow washing
- CD/Abs/Fluorescence measurement

- Up to 192 samples can be loaded
- Small sample volume (~100 µL)

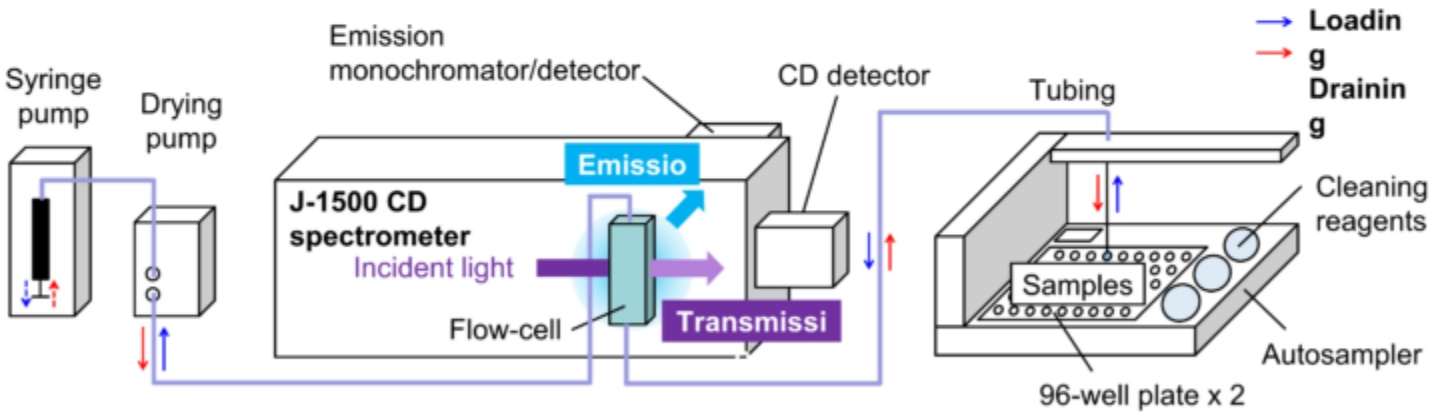


*Fig. 4 Schematic diagram of HTCD Plus system*

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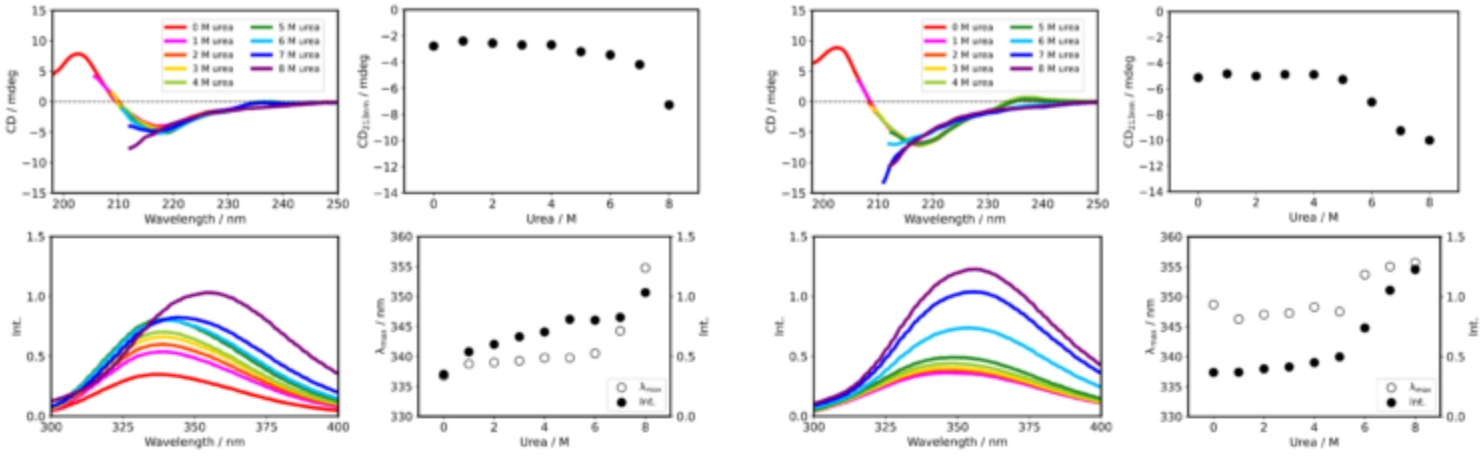
# Results

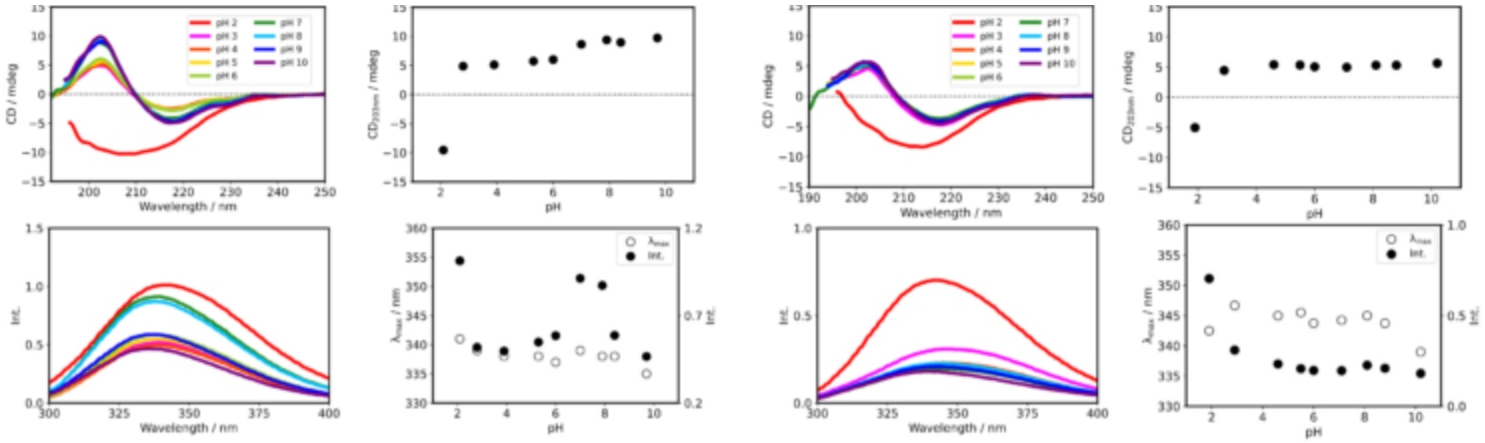
## Urea Concentration

Both Herceptin® and RIABNI™ showed a decrease in CD213nm and an increase in fluorescence intensity as the urea concentration increased. Also, the fluorescence λmax wavelength for the two therapeutic antibodies shifted to longer wavelength as the urea concentration increased.




## pH

Both Herceptin® and RIABNITM showed an increase in CD203nm as the pH increased. However, the fluorescence intensity and λmax showed different behavior for the two antibodies.




Parameters:

• Each sample was loaded four times, with 120 µL of sample used for each measurement. All the spectra were collected at 25ºC.

• Parameters for CD spectrum collection: pathlength = 1 mm; D.I.T. = 2 sec; bandwidth = 1 nm; scan speed = 50 nm/min; averaging over four accumulated scans per sample.

• Parameters for fluorescence spectrum collection: pathlength = 10 mm; D.I.T. = 1 sec; excitation wavelength = 280 nm; data pitch = 1 nm.

• Parameters for flow washing: 1st wash = 1 % Hellmanex® III 250 µL x 1; 2nd wash = ultrapure water 300 µL x 4; 3rd wash = ethanol 300 µL x 2; drying = 60 sec.

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## Conclusion

• Herceptin® and RIABNITM showed a similar dependence on urea concentration, but a different dependence on pH.

• The results indicated the high potential of the HTCD Plus for early-stage screening of therapeutic antibody candidates.

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## Keywords

Circular Dichroism, Fluorescence

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# References

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28600 Mary's Court, Easton, MD 21601 USA • (800) 333-5272 • Fax: (410) 822-7526 • jascoinc.com/applications