

Utilizing A-TEEM™ Technology for In-Process Monitoring of Monoclonal Antibody Production



Application
Note
Life Sciences
FL-2025-06-11

Introduction

Monoclonal antibodies (mAbs) are lab-produced molecules designed to mimic the immune system's ability to recognize and bind to specific targets, such as viruses, bacteria, or cancer cells. Monoclonal antibodies as proteins are composed of amino acids. Some amino acids, specifically tyrosine, tryptophan and phenylalanine are intrinsically fluorescent. This, therefore, allows fluorescence spectroscopy to be used to monitor structural and environmental changes in monoclonal antibodies, making it a valuable tool for their characterization and quality assessment during manufacturing.

Fluorescence spectroscopy is a widely used technique that enables non-destructive, real-time analysis of biological samples with a high level of sensitivity. This makes it ideal for studying cellular processes, protein interactions, and molecular structures. Additionally, it can be used in complex mixtures without the need for extensive sample preparation. For over 30 years, excitation-emission matrix (EEM) fluorescence spectroscopy has been utilized in the literature to characterize or "fingerprint" biological samples. However, in the past, collecting spectra was a time consuming process that required extensive corrections. Advancements in Charge-Coupled Device (CCD) technology and improved correction methods for accuracy issues like the Inner Filter Effect (IFE), have re-ignited interest in EEM fluorescence spectroscopy. The HORIBA Veloci™ BioPharma Analyzer is a 2-in-1 absorbance and fluorescence spectroscopy system, which leverages advanced A-TEEM (Absorbance-Transmittance fluorescence Excitation and Emission Matrix) acquisition technology to capture spectral data in a short

time. With acquisition times between a few seconds to a few minutes, depending on the sample.

In the field of biologics manufacturing, there is increasing interest in adopting novel process analytical technologies (PAT) to support automation. Several studies have proposed EEM fluorescence spectroscopy as a promising PAT tool for this industry. In this paper, HORIBA presents evaluation work from CPI showing A-TEEM as a suitable technique to analyze six in-process manufacturing samples of monoclonal antibodies. This work is an initial step toward implementing this technique as a PAT solution in biologics manufacturing.

Materials and Methods

The manufacturing process of monoclonal antibodies (mAbs) involves several well-defined stages to ensure product purity, safety, and efficacy (see Figure 1). To monitor the process, samples were collected at six key points for analysis with A-TEEM.

Six Key Points for Analysis

1. Cell Culture Media
2. Bioreactor - Protein A load,
3. Protein A - Flow-through,
4. Protein A - Wash I,
5. Protein A - Wash II,
6. Post-viral inactivation

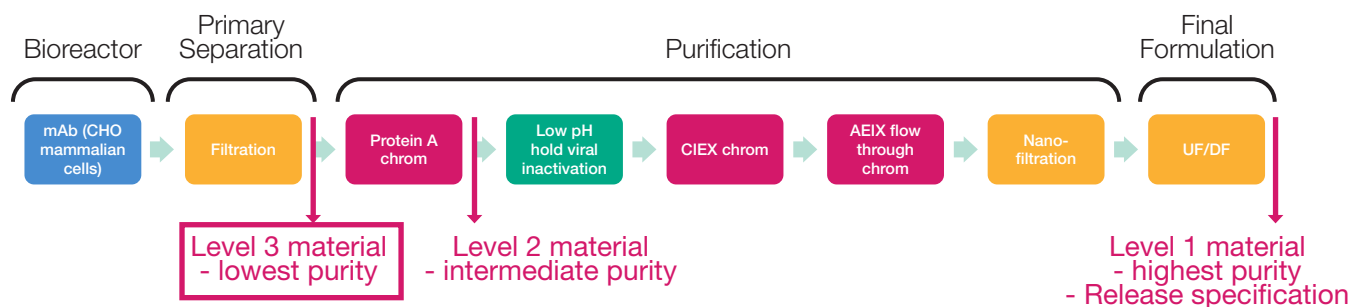


Figure 1. Overview of the mAb Biomanufacturing process including upstream and downstream steps to final product.

Each sample was diluted to achieve an absorbance value of approximately 0.5 au. Excitation-Emission Matrix (EEM) data were collected with excitation wavelengths ranging from 239 nm to 800 nm in 3 nm increments, and emission wavelengths from 250 nm to 800 nm in 5 nm increments.

The integration time was set to 0.01 seconds. Data were corrected for inner filter effect (IFE), as well as for first- and second-order Raman scattering at 16 nm and 32 nm bandpass, respectively, and normalized to a maximum intensity of 1. Parallel Factor Analysis (PARAFAC) was conducted using Solo software (Eigenvector Research Inc., USA).

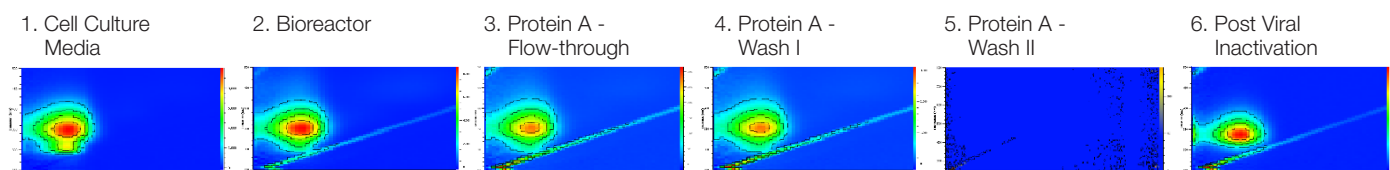


Figure 2. A-TEEM contour plots from the six in-process samples

Data and Results

PARAFAC analysis returned a 4-component model to explain the raw data variance. The model successfully modelled the raw data with over 98% accuracy. Each of the PARAFAC components corresponded to a specific biological fluorophore: tyrosine (Ex. 275 nm, Em. 300 nm), tryptophan 1 (Ex. 278 nm, Em. 330 nm), tryptophan 2 (Ex. 278 nm, Em. 350 nm), and a group comprising vitamins and co-factors (Ex. 356 nm, Em. 455 nm).

Figure 3 displays the modelled emission, excitation, and component loadings for each of the six samples. Notably, the data reveals a red shift in tryptophan emission in the post-viral inactivation (post-VI) purified monoclonal antibody (mAb) sample. This red shift suggests a change in the local environment surrounding the tryptophan residues, which is often used as an indicator of protein folding state.

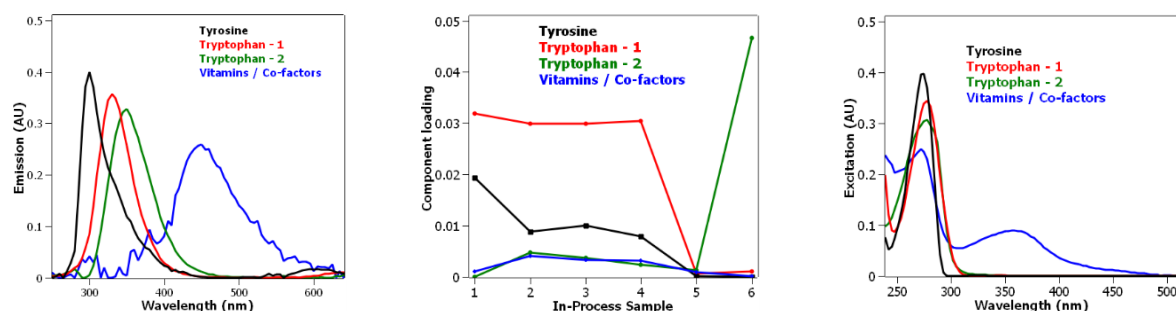


Figure 3. The PARAFAC model Left) Excitation, Middle) Emission, and Right) Component loading of the six In-Process samples

Conclusion

In this study, A-TEEM spectroscopy was employed to characterize six in-process monoclonal antibody samples. As an analytical technique, A-TEEM offers several advantages—it is rapid, easy to implement, highly sensitive, and generates data-rich outputs. These qualities make it well-suited for use either as a standalone tool or in combination with other spectroscopic methods such as Raman, UV-Vis, or infrared spectroscopy. The successful application of A-TEEM technology in the context of monoclonal antibody production highlights its potential as a powerful Process Analytical Technology (PAT) for biologics manufacturing. This work represents an initial step toward evaluating the feasibility of A-TEEM.

Acknowledgements

HORIBA thanks CPI, in particular Dr. Daniel Myatt and Dr. Vicki Linthwaite, for their valuable work in assessing A-TEEM as an analytical tool in bioprocess measurement in the context of the project “Integrating Continuous Technologies for the Rapid Delivery of Cost-Effective Biotherapeutics to Patients,” funded by Innovate UK (project 93825).

References

- 1) Myatt D. et al., “Controlling antibody in-process purification through innovative A-TEEM fluorescence fingerprinting analytics. Poster presented at AIS at AIS, Tours, 22nd June 2023.
- 2) Myatt D. et al., “The Development of Continuous Biologics at CPI.” Talk presented at BPS Europe, Barcelona, 21st March, 2025.

info.sci@horiba.com

www.horiba.com/scientific

USA: +1 732 494 8660
UK: +44 (0)1604 542 500
China: +86 (0)21 6289 6060
Taiwan: +886 3 5600606

France: +33 (0)1 69 74 72 00
Italy: +39 06 51 59 22 1
India: +91 80 41273637
Brazil: +55 (0)11 2923 5400

Germany: +49 (0) 6172 1396 0
Japan: +81(75)313-8121
Singapore: +65 (0)6 745 8300
Other: +33 (0)1 69 74 72 00