Mini Case Session 1: Advances in Process Analytics and Real Time Release Testing (RTRT)

WCBP, January 28, 2026

Co-Facilitators:

Douglas Richardson, Merck & Co., Inc

Qi Zeng, GSK

Susan Park, Genentech, Inc.

Agenda

- Introductions (5 min)
- Scope and Ground Rules (5 min)
- PAT and QbD (5 min)
- PAT Definitions, Case Study & Discussion (25 min)
- RTRT Definitions, Case Study & Discussion (10 min)
- Wrap-up (5 min)
- Resources

Scope & Ground Rules

In Scope

- Focus on process analytics (ie tools and case studies)
- Share experiences with development and implementation of at-line, in-line, on-line PAT.
- Discuss Real Time Release Testing (RTRT) for large molecules including definition, scope, barriers and examples.

Out of Scope

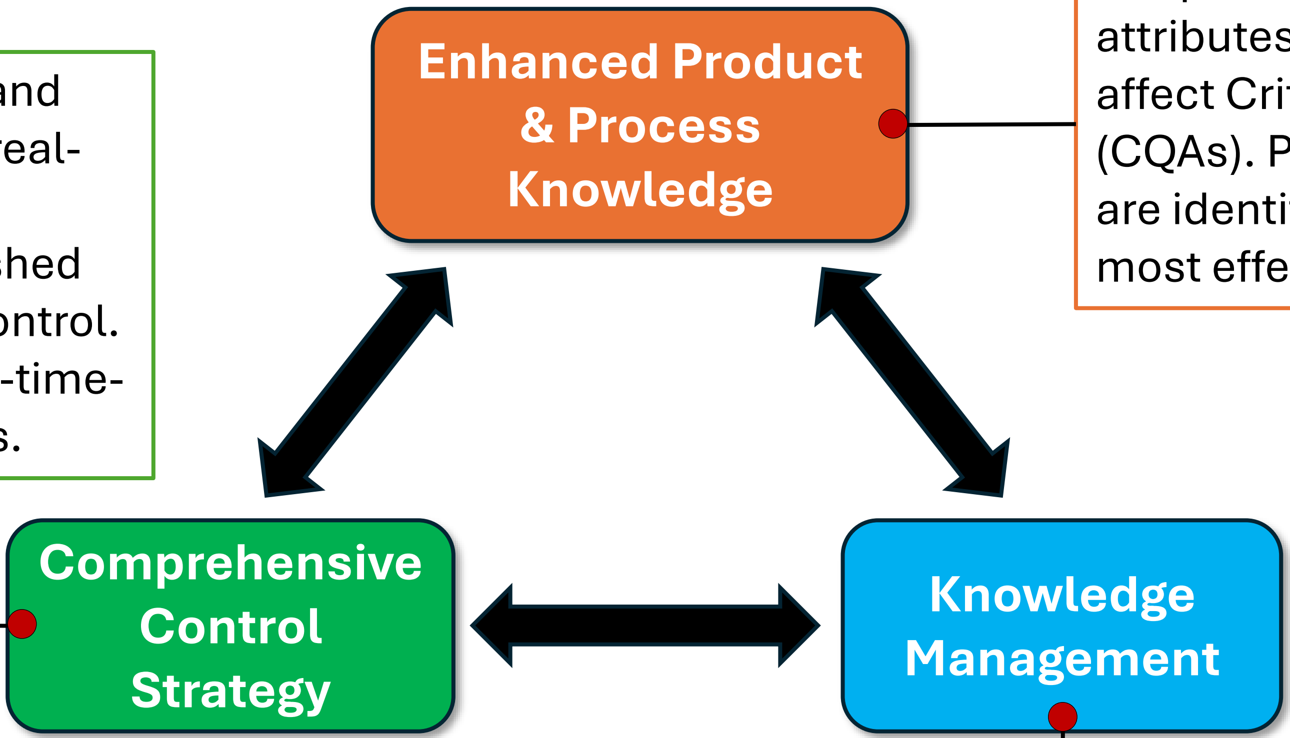
- Process Design
- Quality Systems

Ground Rules

- Raise your hand to speak
- Share Name and Company
- Listen to others
- Share knowledge/examples

PAT as a part of QbD

Monitoring critical quality and performance attributes in real-time to ensure the process remains within the established Design Space or state of control. Enables application of real-time-release testing approaches.



Deep understanding of how material attributes and process parameters affect Critical Quality Attributes (CQAs). Primary sources of variability are identified and controlled at the most effective points.

Essential to have capabilities (IT systems, PQS, etc.) to capture, assess, and share the complex volume of data generated, which supports technology transfer and continual improvement across the product lifecycle

QBD Implementation and PAT Analytics

[Current PAT Landscape in the Downstream Processing of Biopharmaceuticals, Analytical Science Advances, 2025]

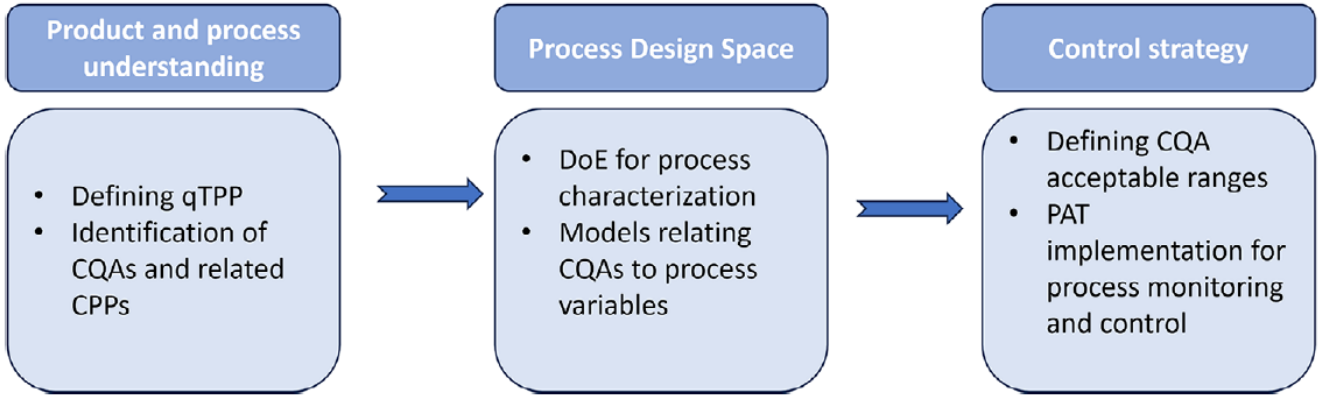


FIGURE 1 | Overview of steps involved in QbD implementation.

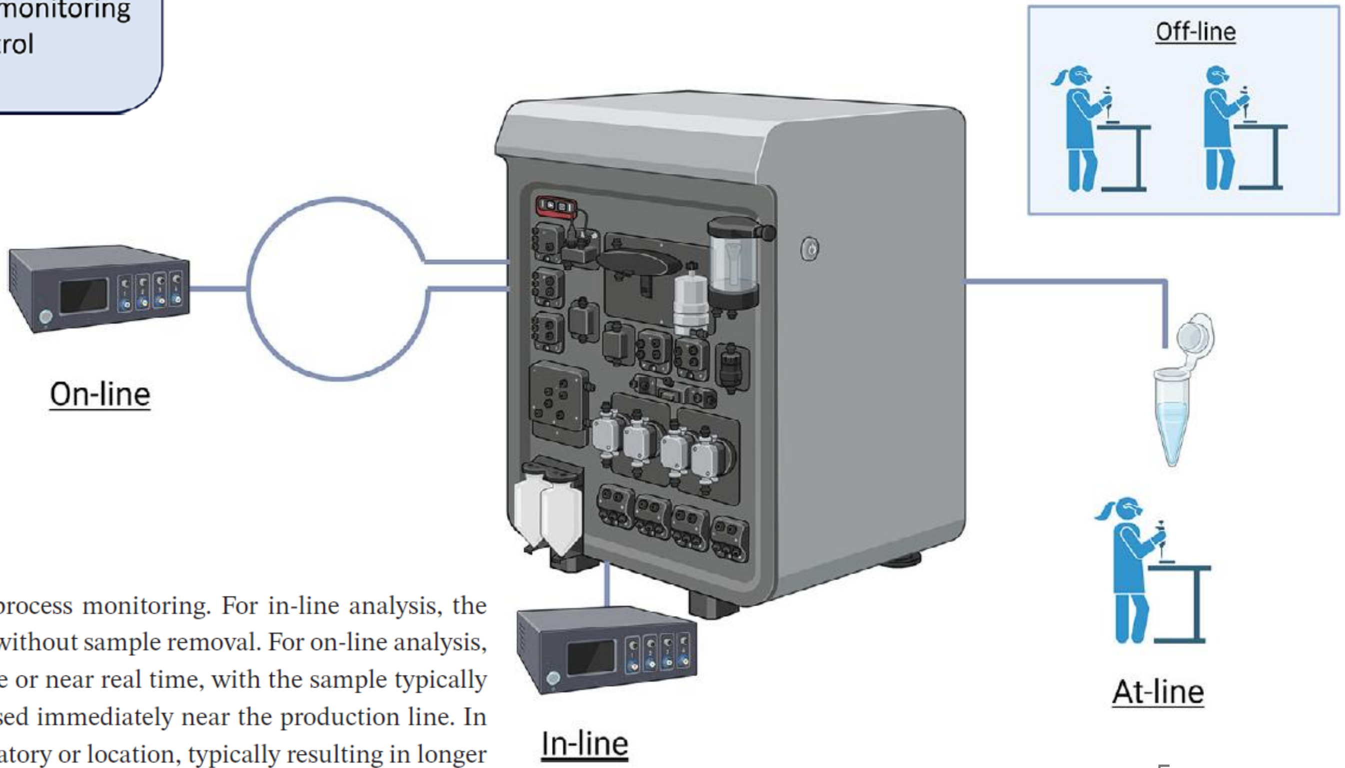


FIGURE 2 | An illustration of in-line, on-line, at-line and offline setups of analytical tools for bioprocess monitoring. For in-line analysis, the analytical tool is directly integrated into the process stream, allowing continuous, real-time measurement without sample removal. For on-line analysis, a portion of the process stream is diverted to an analyser, where measurements are conducted in real time or near real time, with the sample typically returned to the process. For at-line analysis, samples are manually extracted from the process and analysed immediately near the production line. In contrast, offline analysis involves samples being collected from the process and analysed at a remote laboratory or location, typically resulting in longer delays between sampling and obtaining results.

Definitions:

Process Analytical Technology

PAT is a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e. during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality.

Process Analyzers

At-line: Measurements where the sample is removed, isolated from, and analyzed in close proximity to the process stream.

On-line: Measurement where the sample is diverted from the manufacturing process, and may be returned to the process stream.

In-line: Measurement where the sample is not removed from the process stream and can be invasive or non-invasive.

Question: Do you agree with the definitions in the guidance? Could they be improved?

FDA *Guidance for Industry: PAT – A Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance*

PAT Case Study

Millipore®
Expert Pharm/BioPharm Products & CTDMO Services

White Paper

Application of Process Analytical Technology (PAT) in the Antibody-Drug Conjugate (ADC) Bioconjugation Process

Haowei Song, Yiyi Li, Kim Nguyen, Jake Spikes, Mary Ramisetty, Gang Yao, Lisa MacDermott

Song, et al. *Application of Process Analytical Technology (PAT) in the Antibody-Drug Conjugate (ADC) Bioconjugation Process.* Millipore Sigma white paper.

PAT for Inline and Online Monitoring

As shown in **Figure 1**, specific PAT tools can be employed at each of these steps including PendoTECH® (Mettler Toledo), our ProCellics™ Raman technology, as well as PATROL™-UPLC® (Waters) and FlowVPE® (Repligen).

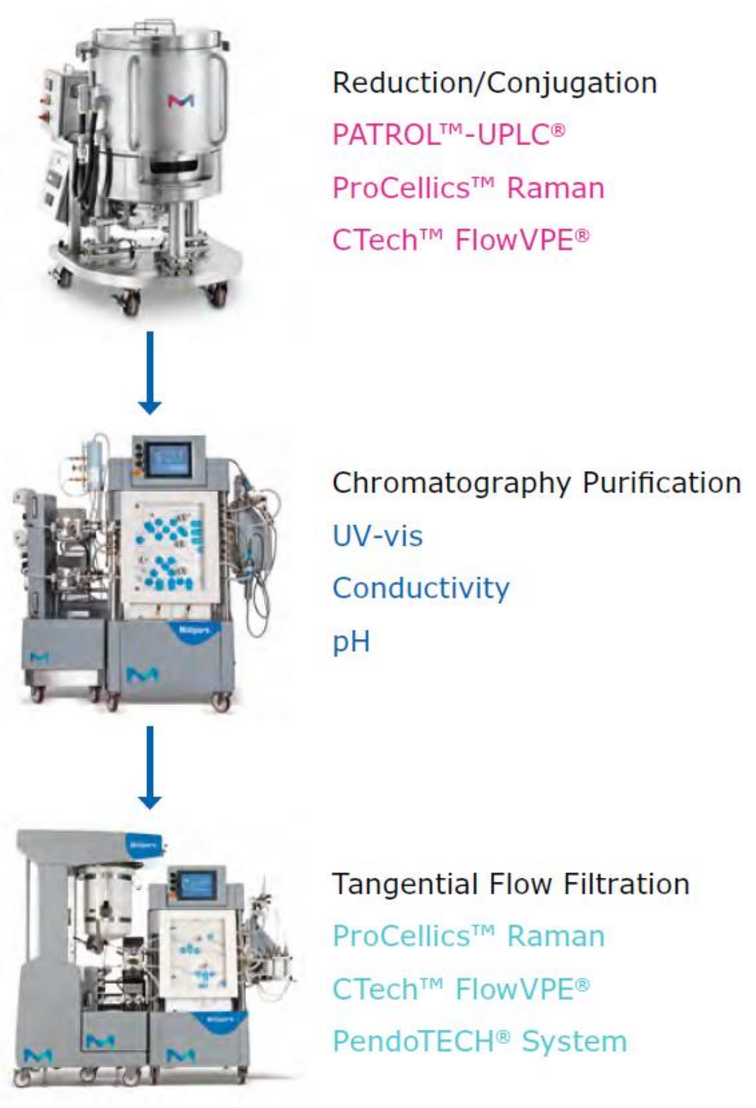


Figure 1.
Each step in ADC bioconjugation requires specific PAT tools.

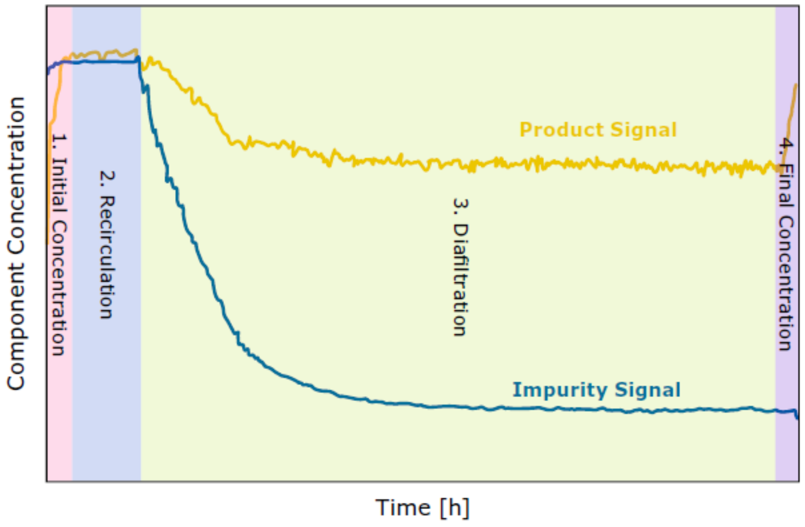


Figure 2.
Inline UV monitoring of a TFF process with FlowVPE®.

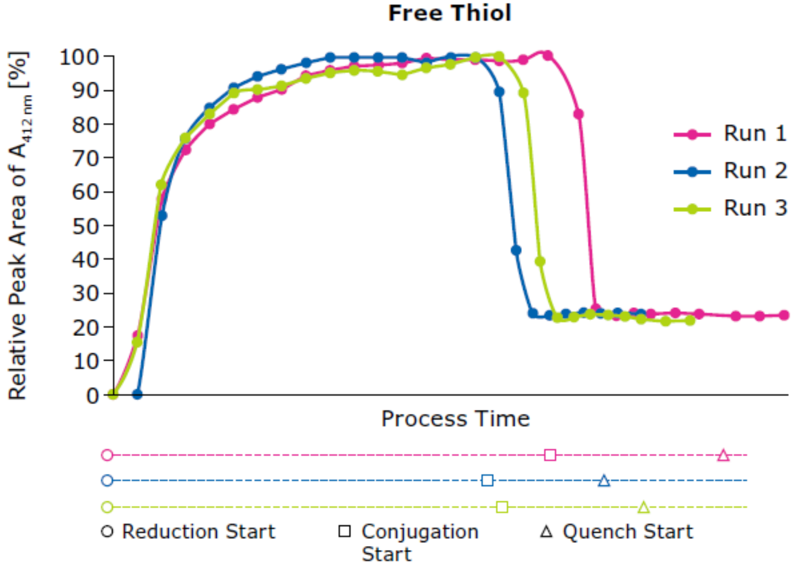


Figure 4.
Real-time online ADC bioconjugation process monitoring.

PAT Discussion Summary

Question: For process analytical technology (PAT) please give technology examples for the following configurations:

- At-line
- On-line
- In-Line

Release Assay/Attribute	PAT	At-Line, On-line, In-Line	Line of sight to RTRT (Y/N)
Protein Concentration			
Aggregation			
Potency			
Micro			
…			

PAT Questions for Discussion

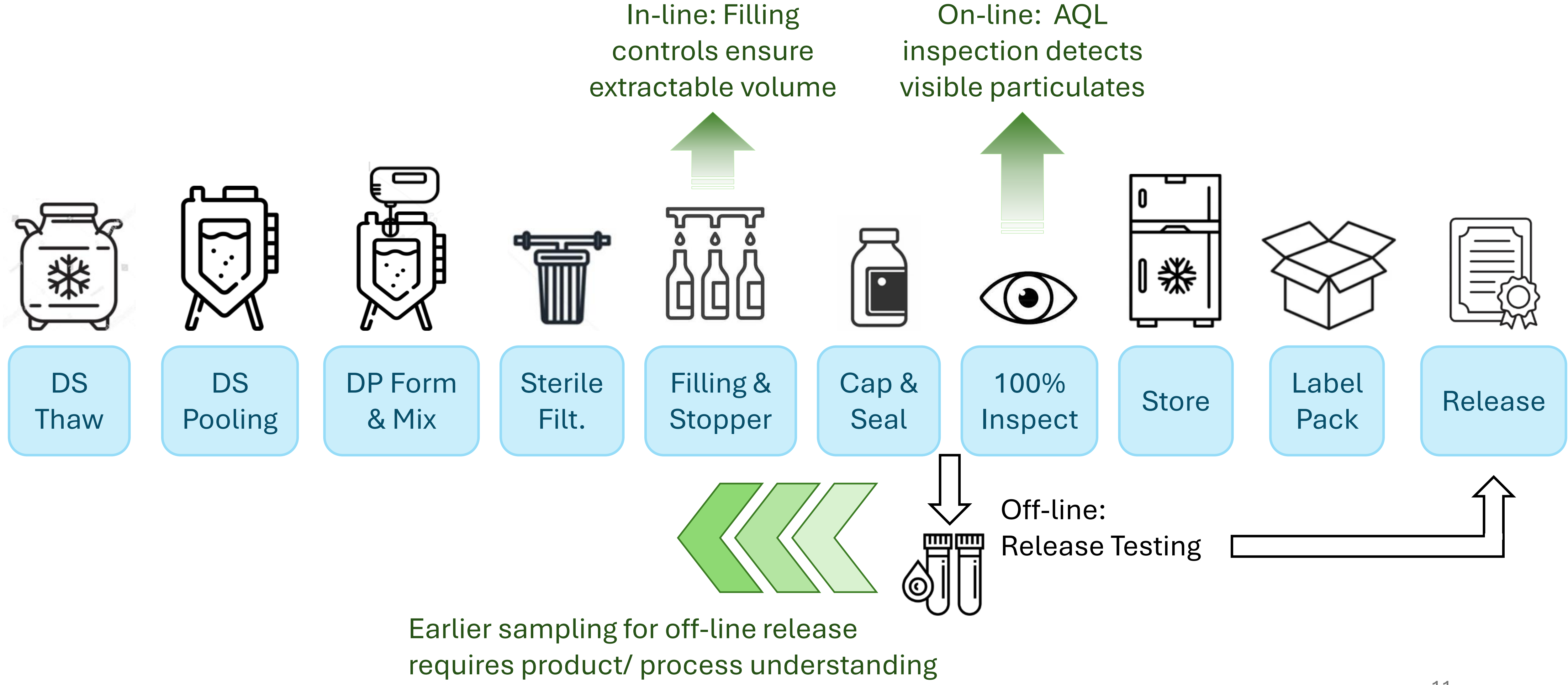
- What are the biggest technology gaps for PAT?
- What are the regulatory challenges for advancing broader PAT implementation?

RTRT (Real Time Release Testing) Definition

Real Time Release Testing (RTRT)

- RTRT is a system of release that gives assurance that the product is of intended quality, based on the information collected during the manufacturing process, through product knowledge and on process understanding and control. (EMA/CHMP/QWP/811210/2009-Rev1)
- The ability to evaluate and ensure the quality of in-process and/or final product based on process data, which typically include a valid combination of measured material attributes and process controls. (ICH Q8(R2))

RTRT Case Study – Drug Product



RTRT Questions for Discussion

- What is the current state of release testing? (i.e. how long)

- What does RTRT mean to you? (i.e. how fast)

- Where is RTRT needed?
  - Raw Materials
  - Drug Substance
  - Drug Product
  - Finished Goods

- What are major barriers to advancing RTRT?

Some Useful Resources

- EMA *Guideline on Real time Release Testing* (EMA/CHMP/QWP/811210/2009-Rev1)
- EMA resources on QbD concepts
- FDA *Guidance for Industry: PAT – A Framework for Innovative Pharmaceutical Development, Manufacturing and Quality Assurance* (September 2004)
- ICH Q8(R2)/ Q9/ Q10/ Q14
- ICH Q8, Q9 and Q10 Questions and Answers (R5) (EMA/CHMP/ICH/265145/2009)
- FDA *Guidance for Industry: Development and Submission of Near Infrared Analytical Procedures* (August 2021)
- FDA *Guidance for Industry & Staff: General Principles of Software Validation* (January 2002)
- *Operational Vision - Adoption of In-line Monitoring and Real-time Release*, BioPhorum Publication, June 2022
- Sathiyapriyan, et al. *Current PAT Landscape in the Downstream Processing of Biopharmaceuticals.* Analytical Science Advances, 2025
- Patel, et al. *Multi-angle light scattering as a process analytical technology measuring real-time molecular weight for downstream process control.* MABS, 2018
- Song, et al. *Application of Process Analytical Technology (PAT) in the Antibody-Drug Conjugate (ADC) Bioconjugation Process.* Millipore Sigma white paper.

Thank you

Q&A

Appendix

PAT Case Study 2

MABS
2018, VOL. 10, NO. 7, 945–950
https://doi.org/10.1080/19420862.2018.1505178

SHORT COMMUNICATION

Multi-angle light scattering as a process analytical technology measuring real-time molecular weight for downstream process control

Bhumit A. Patel [a], Adrian Gospodarek[a], Michael Larkin[b], Sophia A. Kenrick[b], Mark A. Haverick[a], Nihal Tugcu[a], Mark A. Brower[a], and Douglas D. Richardson [a]

[a]Biologics Process Development and Clinical Manufacturing, Merck & Co., Inc., Kenilworth, NJ, USA; [b]Wyatt Technology Corporation, Santa Barbara, CA, USA

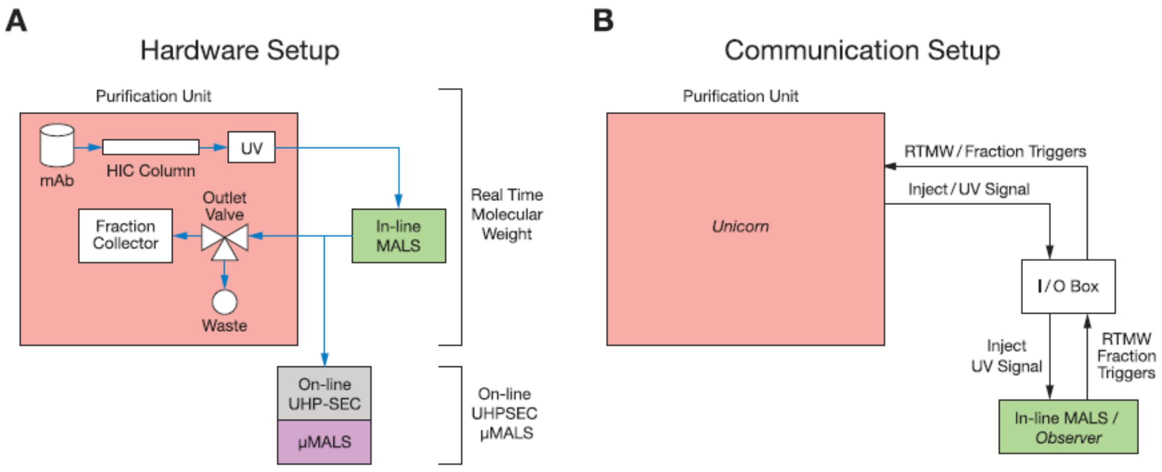


Figure 1. Experimental setup of both hardware and communication of coupling light scattering with purification system.

a). The MALS detector was connected in-line, downstream of the purification unit. HIC purified samples were also sampled downstream of the in-line MALS by on-line UHPLC. On-line UHP-SEC-μMALS served as another application to monitor the fraction and molar mass of aggregates. The samples were separated by analytical SEC and evaluated using a UHP-SEC capable multi-angle light scattering detector (μMALS) and UV signal of the on-line UHPLC as the concentration source. b) The start or inject signal and the UV signal from the purification unit were sent to the in-line MALS via an I/O Box. The UV signal of the purification unit was the concentration source used to calculate M_w of the protein eluted from the HIC column. Real time molecular weight and start/stop trigger signals were sent by the in-line MALS detector to the purification system (via a voltage signal) for fractionation when the measured M_w fell between preset M_w ranges.

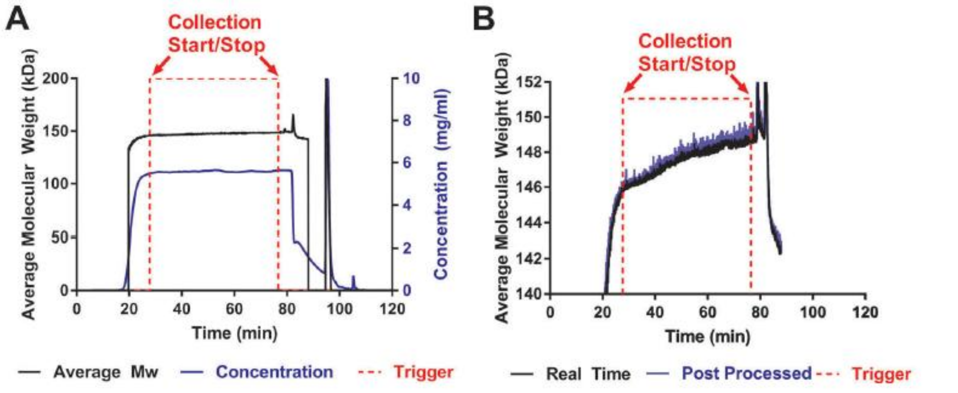


Figure 2. Real time MALS data from a HIC purification.

a). The concentration and M_w signals are plotted along with the trigger signals. Throughout the purification, there is an increase in M_w (—) as aggregates saturate the HIC column and dimers coelute with the monomers. The protein is eluted from the column and passes through both the UV and in-line MALS detectors. The M_w is calculated in < 1 s using both the UV and light scattering signal and a start/stop trigger (- - -) is sent to the purification unit when the protein meets the preset M_w criteria. The concentration is calculated from the UV detector of the purification unit (—). b) No significant differences were observed between the data from real time (—) and post processed modes of analysis (—).

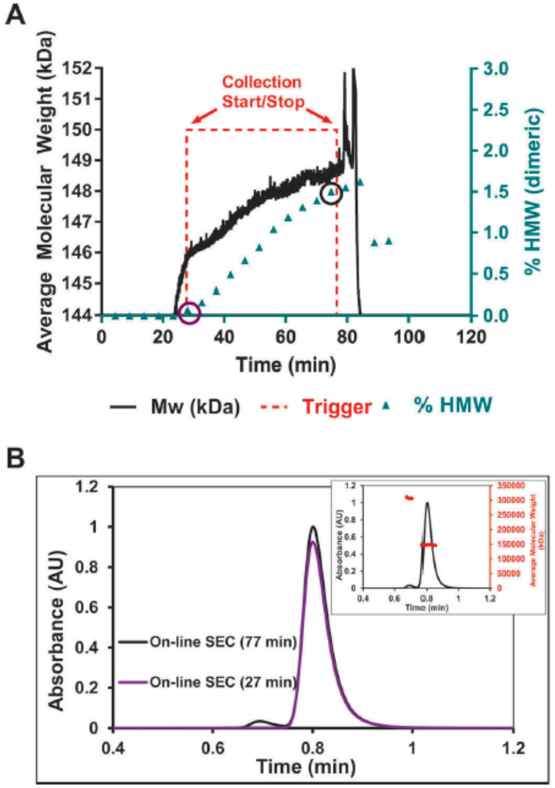


Figure 3. On-line UHP-SEC-μMALS data correlates well with the real time M_w data from in-line MALS.

a) Real time molecular weight (—) shown previously is rescaled to show the increase in M_w of ~ 2750 Da. The HMW content (▲) increases by 1.5% over the course of the purification as determined by on-line UHP-SEC-μMALS. b) On-line UHP-SEC chromatograms of the samples at the start and stop trigger points showed an increase in aggregate content of 1.5%. On-line UHP-SEC-μMALS data confirmed the HMW content to be dimeric (inset). The 1.5% dimeric content correlates to a M_w increase of mAb by ~ 2250 Da. The real time data showed an increase of ~ 2750 Da and is within 0.5% dimeric content of the on-line UHP-SEC-μMALS data.