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White Paper

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

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Process Analytical Technology (PAT) is an important tool for implementing Quality by Design (QbD)[1], which is defined by the International Council for Harmonization (ICH) as a systemic approach to development that emphasizes product and process understanding and process control. Originally proposed by the U.S. Food and Drug Administration (FDA) nearly two decades ago, PAT has since been adopted as an ICH Q8 (R2)[1] guidance. Over the years, extensive work has been done by academic and industry contributors to advance PAT, and it has now become increasingly practical and feasible for real-time applications in biopharmaceutical manufacturing processes.

PAT encompasses several critical aspects that are essential for its successful implementation in manufacturing processes:

- **Timely Measurements:** PAT requires automated sampling and fast turnover times to ensure timely and accurate measurements during the manufacturing process.
- **Handling Complex Matrices:** In-process testing using PAT must be capable of dealing with complex matrices and a broad dynamic range while maintaining high accuracy.
- **Data Analysis:** The large volumes of data generated by PAT require robust data analysis techniques, including statistical methods for data mining and trending.
- **Process Understanding and Control:** PAT is not merely about data collection; it involves understanding the data and translating it into actionable insights for process improvement and control.

PAT offers several advantages that can significantly enhance biopharmaceutical manufacturing:

- **Advancing Manufacturing:** PAT is a key component of automation and smart manufacturing, making it a powerful tool for implementing QbD.
- **Risk Reduction:** PAT helps reduce production risks by enabling better process monitoring and control, thereby enhancing product consistency and minimizing rejections.
- **Efficiency Improvement:** By providing a deeper understanding of the process, PAT can enhance efficiency, reduce overall costs, and improve product quality.
- **Real-Time Product Release:** The goal of PAT is to enable real-time product release in Current Good Manufacturing Practice (GMP) environments, significantly accelerating the production process.

While PAT offers significant benefits, implementation of it into an existing, validated GMP process can be complex, and sometimes costly. It can also be challenging to validate the cleaning process when introducing complex PAT equipment if single-use is not an option. Method qualification in a GMP setting presents a similar challenge as PAT systems often involve a hybrid of analytical instruments, manufacturing equipment, and automation systems.

This white paper describes the application of PAT to gain a comprehensive understanding of the bioconjugation step used in the production of antibody-drug conjugates (ADCs) and to better characterize ADCs and other bioconjugate products. Several PAT tools are described along with case studies demonstrating their application.

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ADC Bioconjugation

A typical ADC bioconjugation process involves the chemical linking of a cytotoxic drug (the payload) to a monoclonal antibody (mAb) through a stable linker. Linking is achieved by targeting specific functional groups on the antibody, such as cysteine or lysine residues for stochastic conjugation or engineered residues to achieve site-specific conjugation.

For stochastic conjugation with cysteine, a reduction step is used to reduce disulfide bonds and form reactive free thiol (-SH) groups on the cysteine residues within the mAb, a critical step for enabling conjugation of the payload. Following reduction and conjugation, the ADC can be purified using chromatography and tangential flow filtration (TFF).

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 provides an example of parallel monitoring of both the product signal and impurity signal during a typical TFF process by FlowVPE®. During the initial concentration, the product signal increases, and both the product and impurity signal remain stable during the recirculation step. In the diafiltration step, there is a significant drop in the impurity signal, with the product signal remaining steady. In the final concentration step, the product signal increases.

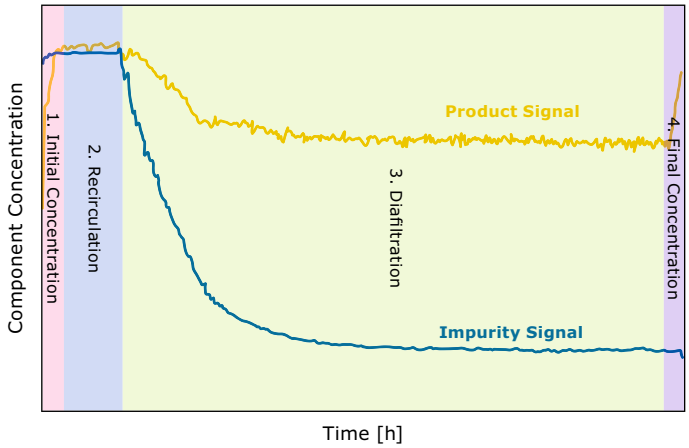

Figure 2.

Inline UV monitoring of a TFF process with FlowVPE®.

The PendoTECH® System is used during process development to provide automated control and real-time monitoring of pH and conductivity during TFF. Inline measurements of pH and conductivity confirm the completion of buffer exchange while the permeate pressure can be automatically adjusted to maintain transmembrane pressure (TMP) at the desired set point.

Raman spectroscopy is another PAT tool that can be used to identify and determine the concentration and physicochemical change of molecules in complex matrices. It is non-destructive and can provide simultaneous, real-time monitoring of several process parameters and quality attributes. The ProCellis™ Raman system has statistical software embedded and is available in both single and four-probe models, allowing flexibility in the number of reactions that can be monitored.

PATROL™-UPLC®, with online sampling and dilution, can also be used for real-time qualitative and quantitative analysis for multiple analytes in complex matrices and as a PAT for the ADC conjugation process.

FlowVPE® is a variable path length, UV-based technology for monitoring protein concentrations over a range of 0.1 mg/mL to 250 mg/mL. During the TFF process, FlowVPE® can be implemented in the feed line to monitor real-time changes in the product, or it can be placed immediately after the TFF membrane to detect any changes post-filtration for TFF process development.

We have developed a PATROL®-SEC method with post-column reaction with DTNB for real-time online monitoring of both the reduction and conjugation processes. For cysteine chemistry, disulfide bonds are first reduced in the mAb, releasing free thiols which are then capped with a drug linker to complete the conjugation process. A UPLC Size Exclusion Chromatography (SEC) column is used to separate the mAb from impurities, ensuring the most accurate quantification of free thiols. Following this step, post-column reaction with DTNB (5,5"-dithiobis-(2-nitrobenzoic acid)) is used, generating an absorbance signal at 412 nm. This approach allows the changes in free thiols to be monitored throughout both the reduction and conjugation process by tracking the absorbance; an increase in absorbance is expected during the reduction phase, followed by a decrease during the conjugation phase.

Case Study: Real-time Online ADC Process Monitoring

In this experiment, performance of the PATROL®-SEC DTMB system was evaluated across different process scales and reactor types using a model ADC. This involved cysteine reduction and maleimide conjugation chemistry, initially conducted at a 1.5 g scale in a 300 mL glass reactor (**Figure 3A**) and subsequently at a 40 g scale in a 10 L single-use reactor (**Figure 3B**).

The process began with the reduction of the mAb, leading to the generation of free thiols. This stage was characterized by a rapid increase in the reaction curve, which gradually slowed down before plateauing. Upon the addition of the drug linker, the curve showed a steep decline due to the drug linker conjugating and capping the free thiols. Notably, the conjugation occurred much faster than the reduction. At the larger scale with a single-use reactor, the kinetic profile was similar and consisted of a rapid initial reaction phase, reduction reaching a steady state, followed by a rapid conjugation.

This study demonstrated that the process was comparable across different scales and different types of reactors. Comprehensive reaction kinetics can be reviewed immediately following process completion, and the progress of the reaction can be visualized in real-time.

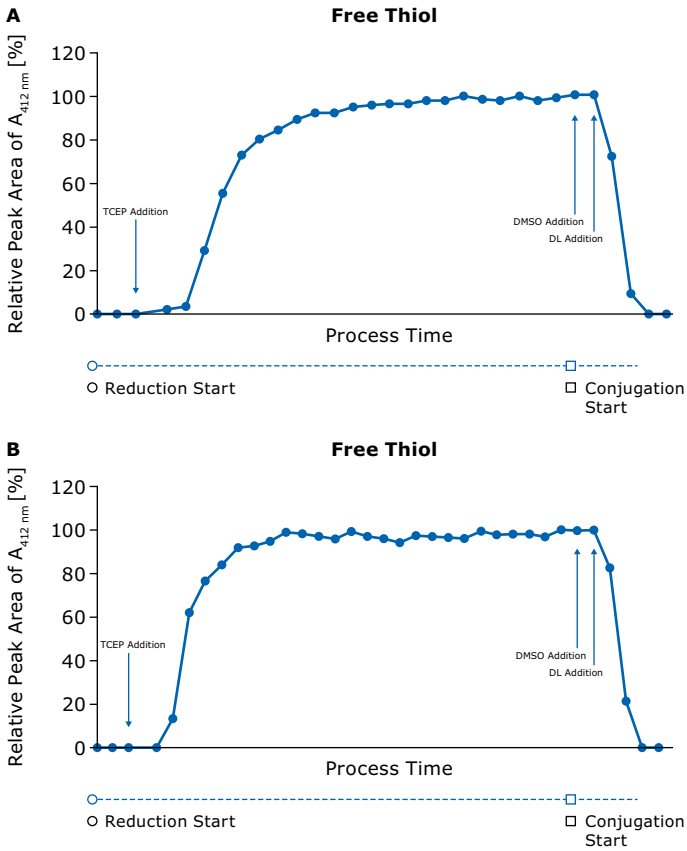

Figure 3.

Monitoring ADC conjugation using the PATROL®-SEC DTNB technique. **(A)** 1.5 g scale cysteine chemistry in 300 mL glass reactor and **(B)** 40 g scale cysteine chemistry in 10 L single-use reactor.

Case Study: Bioconjugation Process Optimization

The goal of the second set of experiments was to explore potential process improvements based on the data generated using the PATROL® system with a clinical ADC. This study also involved cysteine reduction and maleimide conjugation chemistry, with the initial run conducted at a small scale (**Figure 4**, run 1, pink trace). Data from this run revealed that the reduction phase was completed earlier than the initial target time, and the conjugation reaction was finished well before the quenching phase began. These findings suggested that the reaction times could be further optimized.

Building on the insights from the first run, a second run was performed at a similar scale, this time shortening the reduction and conjugation times compared to the initial targets. The objective was to determine whether these adjustments would impact the kinetic profile, drug-to-antibody ratio (DAR), or any other critical quality attributes of the product. The results of the second run, represented by the blue trace in **Figure 4**, showed that the kinetic profiles of both runs were comparable. Additionally, there was no impact on the DAR or other critical quality attributes (data not shown). This confirmed that the process was consistent across both runs and suggested that reaction times could be reduced without compromising product quality.

To further validate these results, the process was scaled to a 10 L single-use reactor for the third run, conducted at 5× the scale of the previous runs. This run is shown by the green trace in **Figure 4**; the kinetic profile remained consistent with those from the first two runs. It should be noted that the timer for the third run (green trace) was not set correctly, and the reduction was executed longer than planned as shown in the figure. This further demonstrated the power of PAT, as any deviations or unexpected changes will be captured and the technology will provide insight and information for troubleshooting if an investigation is needed.

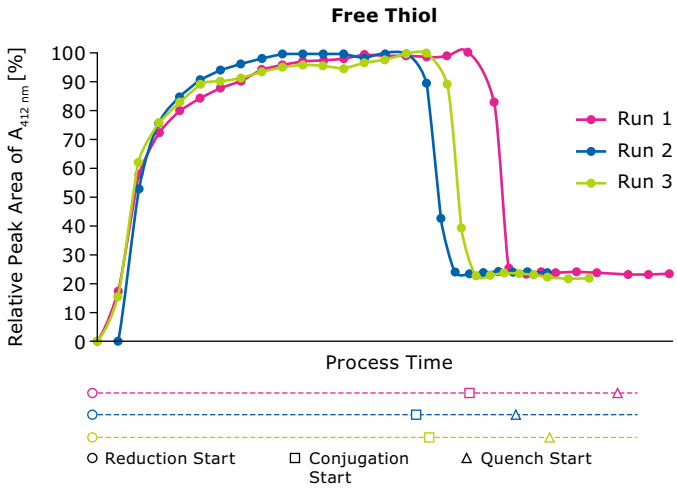

Figure 4.

Real-time online ADC bioconjugation process monitoring.

In summary, the first run was used to identify areas for improvement, which were then implemented and evaluated at small scale in the second run where effectiveness was verified. Finally, in run three, process consistency at a larger scale was confirmed, demonstrating that the shortened reaction times did not compromise the kinetic profile or offline product quality.

The benefit gained from using PATROL® as a PAT in this case study went beyond simply shortening the reduction of conjugation times and overall process times. Reaction kinetics were revealed, allowing process ranges and reaction time targets to be established early in the project. This would not have been possible using conventional off-line monitoring approaches which would have required numerous experiments.

In a GMP environment, batches with a consistent kinetic profile not only demonstrate a robust process but also that the process is under control. There is also increased confidence in product quality before receiving release data from the quality control team.

Case Study: Kinetic Profile Analysis

In the next series of experiments, PAT was used to monitor the kinetic profile of a second clinical ADC. Despite similar chemistry and DAR targets, reaction kinetics differed significantly from those in the previous experiment; in this study, the reduction was much slower, and conjugation was more rapid (**Figure 5**). This experiment underscored the importance of PAT in understanding and controlling complex bioconjugation processes.

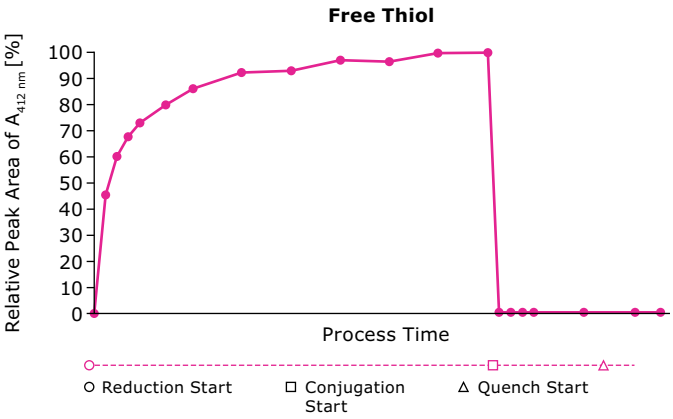

Figure 5.

Real-time online ADC process monitoring showed a slower reduction and faster conjugation.

Conclusion

PAT is a powerful tool that has the potential to reshape bioconjugation processes in ADC manufacturing. By enabling real-time monitoring and process control, PAT enhances manufacturing efficiency, product quality, and overall process understanding. While challenges remain, particularly in GMP environments, the adoption of PAT for QbD is already underway across the pharmaceutical industry. As the industry continues to evolve, PAT will play a pivotal role in shaping the future of drug manufacturing, ensuring that high-quality products are delivered to patients in a timely and efficient manner.

References

1. EMA/CHMP/ICH/167068/2004

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