A Practical Design

Measuring antibody titer is critical in biologics manufacture, and at-line approaches may be the long-awaited alternative to traditional, time-consuming processes

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In recent years, regulators have been encouraging pharmaceutical and biopharmaceutical companies to incorporate the concepts of quality by design (QbD) and process analytical technology (PAT) into drug development and manufacturing processes (1). This should be seen as part of an ongoing drive for scientists to gain as much knowledge and understanding about a new drug as possible (rather than simply collecting the data required for the formal approval process) using a considered set of critical process parameters (CPPs) to subsequently control production.

Now well-established in the 'small molecule world', biopharma has been much slower to adopt these concepts, and, to date, few drugs have been filed based on QbD data. Industry experts have hypothesised that the nature of biologics brings with it more complex quality concerns and a significantly higher number of input parameters that can have an impact on product quality. As a result, the implementation of QbD within biopharma calls for a significant level of detailed information gathering.

Identifying a parameter as important, such as immunoglobulin G (IgG) titer, creates a requirement for reliable detection. Therefore, the next challenge is identifying analytical instrumentation that can deliver the necessary insights in a way that provides consistency in methodology – or at least a demonstrable correlation of results – as you move from research to production. Traditional techniques, as well as innovative methodologies, must be assessed and applied on this basis.

New Approaches, New Challenges

The ICHQ8 provides us with a well-accepted definition of QbD and a series of insightful guidelines: "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding based on sound science and quality risk management" (2-5).

Understanding clinical characteristics and desired product performance from early in the development process is fundamental to using QbD and PAT to drive development; criteria are set as the goals for product formulation and process development.

Critical quality attributes are identified and set in a quality target product profile. Critical material attributes and CPPs are recognised during product development, and their link to product quality and safety are understood. The final elements of QbD bridge into PAT and relate to process capability and continuous improvement. In addition to desirable goals such as process efficiency, real-time release, and more consistent product, the promise for drug developers is that by capturing all the relevant elements in this structured, risk-assessed way, they can demonstrate to regulators that they know and can control what matters. Importantly, this can lead to more flexibility in the regulatory process.

However, such an approach needs significant investment in resources at very early stages of product development, where it is often far from clear if the drug candidate will be safe and efficacious in later clinical trials.

Back to Basics

Against this complex backdrop, it is helpful to explore a straightforward example of how an identified parameter can be translated into a useful, actionable control point. Protein titer (expressed in g/L) is considered the primary benchmark for characterising upstream manufacturing efficiency, with higher titers generally indicating that more of a desired product is manufactured using the same or less amount of fluid or filled bioreactor volume. With improvements in, for example, expression systems, cell lines, culture media, and media optimisation, as well as better bioprocessing equipment, average commercial scale titers have increased significantly over time and are predicted to rise further by 2023 (see Figure 1) (6).

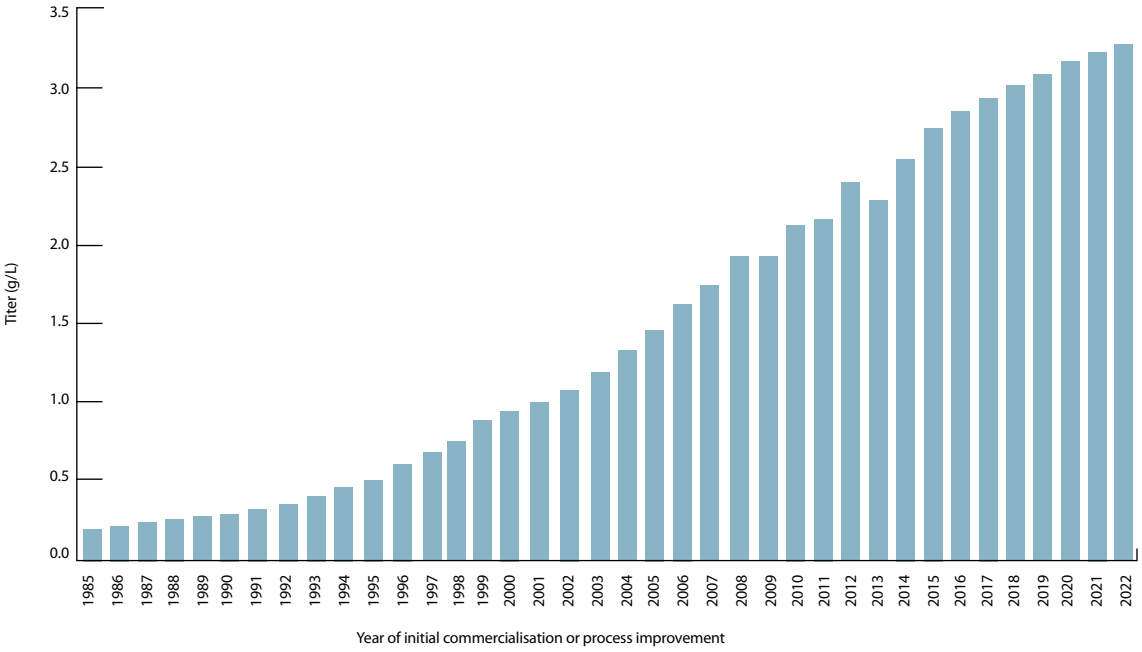

Figure 1: Average commercial-scale titers, 1985–2022

Source: Available data on production trends back to 1985 and forward to 2022, including BioPlan's 11th Annual Report survey data for 2006-2014

A toolbox of analytical methods has been applied to monitor the product quality at various stages of biopharma development. However, it is recognised that there remains a deficit of technology to provide consistent, transferrable methods and data, from research through development to production.

For example, traditional SDS-PAGE and western blot are still considered valuable in R&D, but the matrix complexity and low analyte concentration prevent SDS-PAGE from being a practical analytical method at production scale. On the other hand, traditional slab gel western blot, often associated with laborious manual operations, does not offer sufficiently accurate and precise quantitation or desired turnaround time and sample throughput to meet industrial standards and requirements. At best, these techniques have a role for confirmatory testing. Other analytical tools for titer include immunoassay, bio-layer interferometry (BLI) – an optical quantitation technique – and high-performance liquid chromatography (HPLC). Today, HPLC with Protein A affinity capture is the most used method for titer measurement at production scale. It is considered the 'gold standard'.

The technique offers many of the desirable characteristics for titer measurement; it is rapid and highly specific. However, instrumentation is complex, method development and validation are required, and it is challenging for inexperienced operators to achieve consistent results. As a consequence, HPLC capability is rarely accessible at-line, which makes measurements at multiple stages of the bioprocess challenging and reduces the ability to use the measurement as an actionable control point.

Real-Time Benefits of Actionable Data At-Line

Recent work has compared a novel protein analyser with these current methods. The protein analyser uses a trap-and-elute technology to obtain rapid in-line measurement of antibody titers from stirred bioreactors. The amount of IgG in a cell-free sample is quantified, providing accurate results with an easy-to-use interface that requires minimal operator training.

In one study, assay linearity and accuracy of the protein analyser was compared with HPLC and evaluated over a measurement range of 0.1 to 6.5g/L using a bioreactor spiked with bovine serum albumin (BSA) and human IgG (7). Calibration curves were generated using a human IgG control standard and showed excellent fit (R^2) of linear regression across the calibration range (see Figure 2, page 8). The extended dynamic range allows for extrapolation to higher titer values, which is particularly useful given that many bioprocessing systems are now producing up to 10g/L in fed batch processes (8-10). The correlation of the protein analyser and HPLC titer measurements is shown in Figure 3 (page 8), with excellent agreement across the titer range. The measurement results were unaffected by changing BSA levels

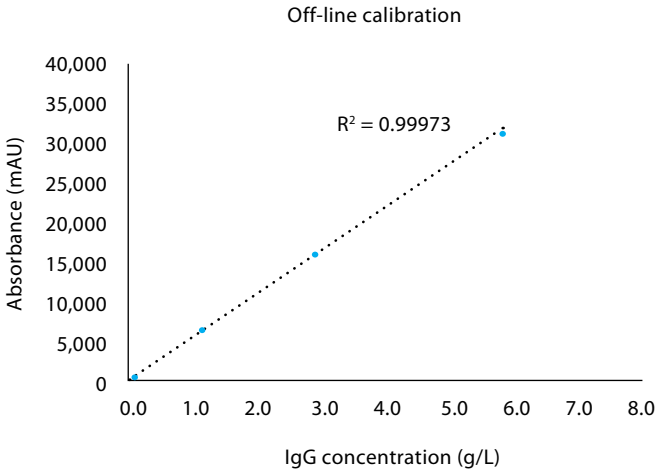

Figure 2: Calibration curves generated using a human IgG control standard – standard measurements at 0.1, 1.0, 2.5, and 5.0g/L were used to generate calibration curves for the protein analyser and HPLC

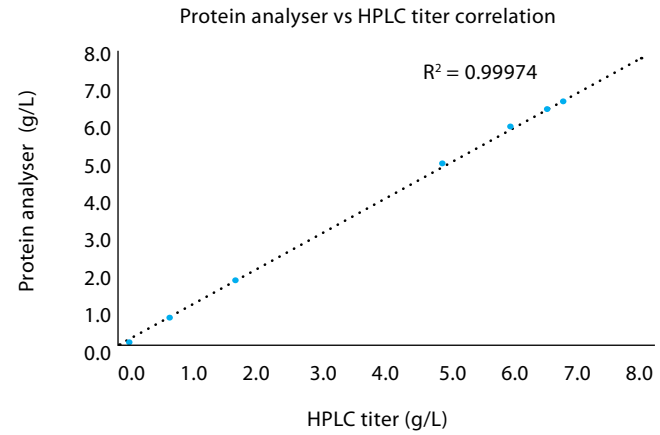

Figure 3: Correlation of protein analyser and HPLC titer measurements

up to 2.5% (v/v) and demonstrate the successful application of an automated, at-line protein analyser for titer measurement.

Titer measurements were completed in under five minutes, and excellent intra-assay precision (CV less than 3%) was observed. A linear response was demonstrated from 0.1 to 6.5g/L without calibration or sample dilution.

Moving On-Line

In addition to the above at-line assessment versus HPLC, the protein analyser was also compared to BLI. In this separate study, a discrete on-line sampling system was programmed to automatically withdraw a user-defined volume of cell-free sample from the bioreactor containing a stable CHO cell line, expressing a humanised IgG antibody (11). The sample was then delivered to the protein analyser system in real time at six-hour intervals over 19 days. The antibody titer of the cell-free sample was analysed via a trap-and-elute separation and compared to off-line measurements using BLI and HPLC (see Figure 4).

Titer values up to 2.4g/L were measured without dilution using the protein analyser, and the results showed that the range, accuracy, and robustness of the chromatographic system for at-line measurement of monoclonal antibody titer were comparable with off-line measurements using BLI and HPLC. Real-time measurements with the protein analyser could potentially enable process controls based on a titer. The analyser streamlines the method development and eliminates the trial and error associated with BLI and other analyses used today. It enables single titer measurements on demand without requiring economies of scale.

In the drive for faster drug development and more efficient production of biologics, the principles of identifying and measuring specific parameters that indicate scientific understanding and/or monitor product and process quality are becoming embedded in the industry. The QbD approach and PAT framework will only increase in importance. As researchers investigate analytical methods that can provide the

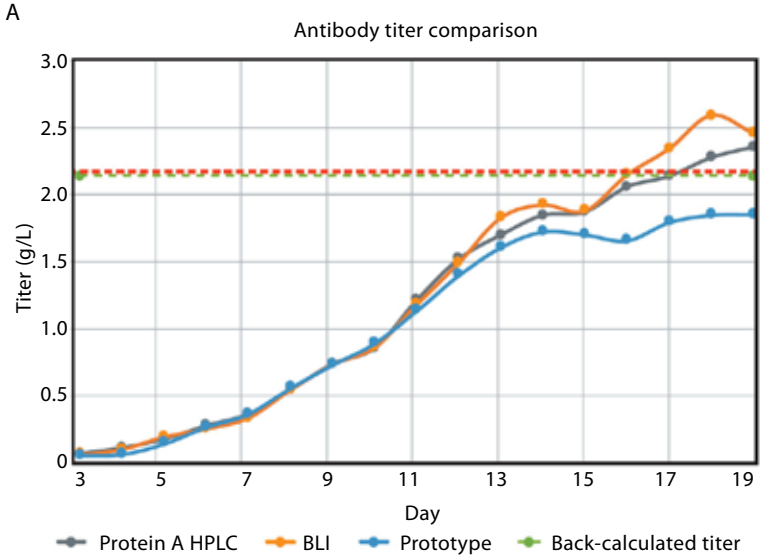

Figure 4: Comparison of real-time antibody titer with off-line antibody titer measurements. Average of three measurements per day plotted for off-line HPLC and BLI analysis; protein titer at harvest was back-calculated following Protein A purification

data that is needed, a new assessment of the suitability of a technique to provide actionable information – either at-line or on-line – becomes of significant importance. The development of novel systems that provide this practical utility, such as the protein analyser, indicate a clear way forward.

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