Innovating the mAb Characterization Process

A disruptive approach to characterizing mAb charge variants

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INTRODUCTION

In the development of protein-based biopharmaceuticals, genetic variations and post-translational modifications (PTMs) give each molecule a "personality," and understanding these unique characteristics drives the development, manufacturability, and analytical methods used to monitor and control the quality attributes and testing requirements. One such quality attribute that requires routine analysis is a molecule's charge heterogeneity profile. When developing the process to produce the drug using living cells to express the protein, care must be taken to ensure that the conditions, cell line, or process itself isn't introducing unwanted variation into the drug molecule.

Because testing the characteristics of charge variants via chromatographic or electrophoretic methods requires extensive work, these actions are often delayed until the latter phases of drug development. As biopharma companies seek the most viable candidates, the ability to rapidly analyze proteoforms can result in significant time savings during the research and development process.

Here we discuss the challenges presented to researchers in such a situation, as well as a case study that describes how collaboration with a novel and disruptive technology enabled FUJIFILM Diosynth Biotechnologies to overcome unexpected results and bypass months of laborious testing.

ANTIBODY THERAPEUTIC TRENDS

Monoclonal antibodies (mAbs) have become one of the world's most frequently researched protein-based biotherapeutics. There are currently 141 mAbs approved or in regulatory review in the United States and European Union.[1] Still, several hundred more are currently in various stages of clinical studies.[1] In 2022, 135 antibody therapeutics were present in late-stage clinical studies across various therapeutic areas, including 61 cancer, 54 non-oncology, and 20 COVID-19 indications.[1] mAbs are also an important research topic in scientific literature. In the past 10 years, 150 peer-reviewed scientific articles have

been written on mAb characterization.[1] In 2023 alone, this topic was featured in 50 poster presentations at the American Society for Mass Spectrometry (ASMS) Conference.[1]

Corresponding to the increase in mAb research is the addition of engineered antibody fragments, bispecific and multispecific antibodies to better treat disease. Introducing new modalities into the pipeline adds another dimension of characterization that has to be met with speed and confidence. With these new antibody modalities the complexity of the charge profile likely increases, which will require more advanced tools to rapidly characterize each molecule (**FIGURE 1**).

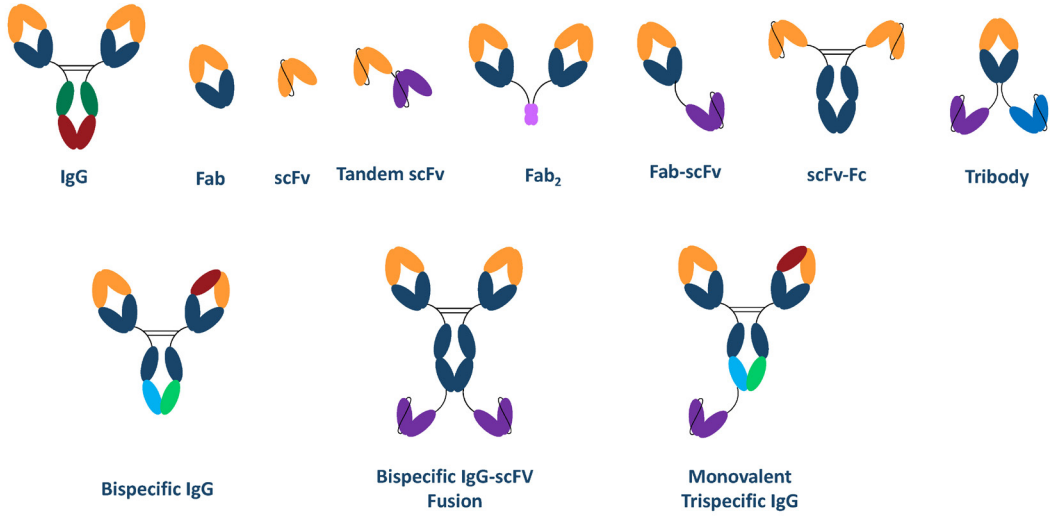
INTACT mAb ANALYSIS CHALLENGES

mAb characterization continues to provide novel opportunities for discovery. Genetic variations and PTMs lead to diverse molecular forms, and a single mAb molecule exhibits numerous common modifications. This results in an array of charge-based variants to analyze, as even extremely minor disparities can be observed during intact charge variant analysis.

Cell-line development groups are tasked with selecting the best clone to move forward into development. They use icIEF to assess the heterogeneity of the protein a particular clone is producing. However, they don't know the identity of each peak, so they rely on previous knowledge and make a risk-based decision on how to move forward. The existing methodologies available to characterize and identify variants typically take months and can create a bottleneck in development timelines. The current workflow involves monitoring charge variants using imaged capillary isoelectric focusing (icIEF) or IEX-LC. These widely accepted approaches increase the number of resources required to obtain peak identification and could introduce artifacts such as oxidized species due to the movement between technologies, as well as extensive sample manipulation. Additionally, separation during the LC process may not accurately represent the icIEF-UV profile. Using electrophoretic methods in process development or in QC testing necessitates the development of an orthogonal IEX-LC method and peak correlation to improve sample data.

These challenges have the practical implication of pushing the appropriate characterization of mAbs to later stages of development, creating a knowledge gap in early-stage research. Existing knowledge is utilized to risk-assess the need for more advanced characterization. The risks of using such

FIGURE 1: Antibody therapeutics are becoming more complex.



assumptions are multiplied when an unexpected variant is present, but the image electropherogram appears like other normal map profiles.

CASE STUDY: AN INNOVATIVE TECHNOLOGY APPROACH

Recently, FUJIFILM Diosynth Technologies encountered an unexpected result during a cell line development project. The cIEF profiles showed unexpectedly high levels of acidic components. The initial efforts for characterization used N-linked glycan methods and peptide mapping with LC-MS/MS. Glycan methods did not indicate an increase in insulative glycoforms, and additional PTMs were not identifiable with peptide mapping. To get to this point, over a month of modifying cell culture conditions, performing small-cell process development studies, and analyzing the IEF imaging was required.

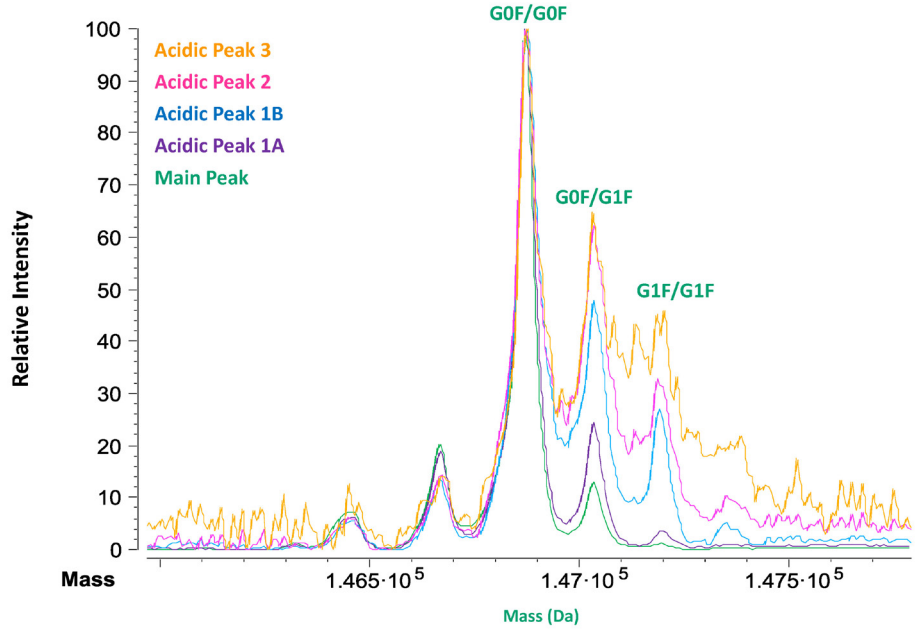
The unexpected results made it challenging to approach the identification of these variants. The development project's stage required a faster solution than in-depth fractionation would allow, and knowledge gaps did not permit predictions based on prior understanding.

The decision was made to partner with SCIEX and leverage the capabilities of the Intabio ZT system. The Intabio ZT system combines charge heterogeneity analysis and molecular mass identification into a single platform solution for comprehensive separation and identification of charge variants.

The Intabio ZT system provides the separation and identification of mAb charge variants without the protein scale-up and fractionation required in standard methods, reducing the process from weeks to minutes by coupling icIEF separation with high-resolution MS identification using the ZenoTOF 7600 system into a single workflow. **FIGURE 2** illustrates the separation among peaks of the acidic variants, indicating the glycation of lysines.

Introducing the integrated platform of icIEF with mass spectrometry detection acted as an answer to the unexpected

FIGURE 2: Acidic variants are mostly glycated species.



acidic component levels. **FIGURE 2** shows the direct characterization of resolved proteoforms enabled by the Intabio ZT system with an accurate determination of the various species' molecular weights. After identifying that the acidic species were changing in mass by +162 Da, it became likely that the pI-dependent shift was due to lysine glycation, resulting in less basic, more acidic proteoforms.

A single icIEF-UV/MS experiment mitigated several months of inaccurate hypothesis formulation and prolonged experimentation.

As shown in **FIGURE 3**, the analytical approach enabled a direct characterization of resolved proteoforms with an accurate determination of the various species' molecular weights. The Intabio ZT system also allowed the FUJIFILM Diosynth Biotechnologies team to understand why the glycated species was not shown in the original peptide map and thus modify methods.

IMPLICATIONS FOR MAB DEVELOPMENT

The data obtained from the evaluation of the Intabio ZT system is promising and demonstrates that the technology can be a disruptive innovation in the development of biotherapeutics. A single icIEF-UV/MS experiment mitigated several months of inaccurate hypotheses formulation and prolonged experimentation. The rapid insights provided by the system identified the cause of the change in charge profile and allowed targeted characterizations to replicate and confirm the findings.

Earlier assessment of molecules provides valuable insights into the manufacturing developability of molecules, reducing the need for unnecessary experimentation. The Intabio ZT system made in-depth characterization of the biopharmaceutical product charge variants significantly more efficient and potentially reduced the timeframe required for early-phase clinical development.

REFERENCE

1. Adams, Greg, Barker, Jason, Mack, Scott, Ostrowski, Maggie. A Disruptive Approach to the Characterization of mab Charge Variants During Process Development. Presented at: *71st ASMS Conference on Mass Spectrometry and Allied Topics*. June 4-8, 2023. Houston, TX.

FIGURE 3: Analysis on the Intabio ZT system and ZenoTOF 7600 system.