Rapid monitoring of monoclonal antibody quality attributes in non-purified perfusion CHO cell culture using ZipChip®-MS

Application Note

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Abstract

Rapid and accurate characterization of monoclonal antibody (mAb) quality attributes during bioprocessing is critical for ensuring product consistency and efficacy. This study demonstrates the use of the ZipChip® microfluidic capillary electrophoresis system coupled with mass spectrometry (ZipChip-MS) for "dilute-and-shoot" analysis of mAb samples from perfusion CHO cell culture bioreactors. With no pre-purification, ZipChip-MS enabled quantification of glycosylation profiles and charge variant distributions, as well as mass confirmation at mAb concentrations as low as 0.4 g/L. The platform facilitated time series analysis of critical quality attributes throughout the culture duration and supported batch variability assessments. The data also showed that cell retention filtration has no significant impact on measured attributes of the mAb harvested from the permeate.

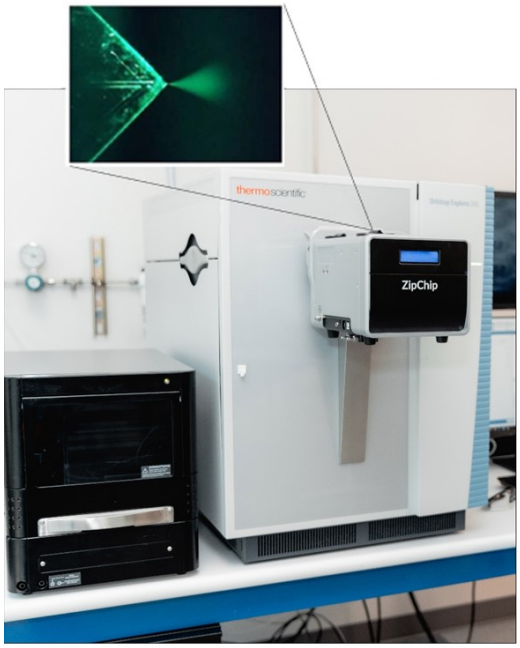

Figure 1. ZipChip Interface on Orbitrap Exploris 240 Biopharma mass spectrometer

Introduction

Monoclonal antibodies (mAbs) are complex biotherapeutics whose efficacy and safety depend on critical quality attributes (CQAs) such as glycosylation and charge heterogeneity. Traditional analytical workflows often require extensive sample preparation, including purification and concentration steps, which can delay feedback and increase process development timelines. The ZipChip-MS method offers a simple alternative to capture multiple CQAs in a streamlined workflow.

The ZipChip System is a microfluidic analysis device that seamlessly integrates high-resolution capillary electrophoresis (CE) with electrospray ionization (ESI) for powerful mass spectrometry (MS) characterization of biologic samples. Previous studies have demonstrated the ZipChip charge variant analysis method with on-line MS characterization (CVA-MS) for in-depth characterization of purified drug substances and its comparability to other techniques.[1,2,3] In this study, spent media samples were taken both directly from the bioreactor and after cell retention filtering. The samples were analyzed without further purification using the ZipChip CVA-MS method to characterize the expressed

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mAb product. Using this method, multiple CQAs are monitored with a single assay: charge variant profile, molecular mass confirmation, and post-translational modifications (PTMs) inducing charge and glycoform heterogeneity. This study evaluates the capability of ZipChip-MS to monitor CQAs directly from perfusion CHO cell culture samples, enabling rapid insights into bioprocess performance and product quality.

Materials and Methods

Perfusion CHO Cell Culture

Eppendorf 3 L water jacketed, autoclavable glass vessels equipped with one pitched blade impeller were used in conjunction with BioFlo 320 Controllers (Eppendorf) for CHO-GS cell cultivation. The agitation was set at 200 rpm. Temperature was maintained at 37°C and pH was controlled at 7.0 ± 0.2 using CO_2 and base addition. Dissolved oxygen (DO) was supplemented via macro- and micro-sparge throughout the process to meet the oxygen demand at high cell densities. Perfusion was initiated on day 3 at a perfusion rate of 1 vessel volume per day (VVD), then increased to 2 VVD on day 5, and finally 2.5 VVD on day 6 through the remainer of the perfusion process (day 15). Glucose was continuously fed to maintain concentrations above 4 g/L. Cell retention was achieved using the XCell® ATF 1 Device (218 cm^2, 144 mL/min cross flow, 2000 s^{-1} shear rate, 5.7 LMH filtrate flux at 2.5 VVD) connected to an XCell Lab Controller. Daily samples were collected from both the bioreactor and the ATF filter permeate line for analysis.

ZipChip-MS

Sample preparation: The ZipChip Charge Variant Analysis Kit (Repligen) was used for sample preparation and analysis. Bioreactor samples were collected in a centrifugal tube and centrifuged for 10 min at 1000g. The supernatant was collected and stored at -80°C until analysis. XCell ATF permeate samples were drawn with a syringe from the harvest line and directly stored at -80°C until analysis. For CVA analysis, samples were diluted 50x with Charge Variant Analysis sample diluent.

Data collection and analysis: Samples were analyzed using a ZipChip interface coupled to an Orbitrap Exploris 240 Biopharma mass spectrometer (Thermo Fisher Scientific). Charge variant analysis was conducted using a high resolution chip for native protein analysis (HRN chip) and the ZipChip CVA kit. The ZipChip and MS acquisition methods are provided in Table 1 and Table 2, respectively.

Data were visualized and peak areas were generated using QualBrowser (Thermo Fisher Scientific). Mass spectra were processed using BioPharma Finder 5.0 (Thermo Fisher Scientific) and UniDec Universal Deconvolution of Mass Spectra software (University of Oxford).

Table 1. ZipChip settings

Parameter	Value
Global Settings	
Sample Volume	20 µL
BGE Refresh Rate	Every 1 line
Method Settings	
Field Strength	500 V/cm
Chip	High Resolution, Native (HRN) part no. 850-00052
BGE	ZipChip CVA
Injection Volume	1 nL
Pressure Assist Start Time	0.0 min
Analysis Time	14 min

Table 2. Orbitrap Exploris 240 settings

Parameter	Value
Method Settings and Global Parameters	
Method Duration	14 min
Ion Source Type	ESI
Gas Mode	Static
Sheath Gas Flow Rate (arb)	2
Ion Transfer Type Temp.	300°C
Pressure Mode	Standard Pressure
Expected Peak Width	5 s
Advanced Peak Determination	FALSE
Full Scan	
Orbitrap Resolution	30k
Scan Range (m/z)	2000–8000
RF Lens	150%
AGC Target	100%
Max Injection Time Mode	Auto
Microscans	3
Data Type	Profile
Polarity	Positive
Source Fragmentation	Enabled
Energy	135 V

Results

The ZipChip CVA-MS method successfully detected and quantified mAb CQAs without prior purification for titers as low as 0.4 g/L (measured in the bioreactor and XCell ATF permeate on day 6 after inoculation). Charge and glycoform heterogeneity, mass confirmation, and PTMs were measured from the resulting data. Figure 2 depicts ZipChip CVA-MS data from day 12 of bioreactor run #179, showing the charge variants detected (A) and the mass spectrum with identified glycoforms (B). Based on mass and electrophoretic mobility shifts between charge variant peaks, the basic variants were found to be caused by C-terminal lysine clipping and C-terminal proline amidation. The acidic variants were primarily due to deamidation, glycation, and complex glycans containing sialic acids. A total of 13 glycoforms were identified by mass, including partially glycosylated mAbs, afucosylated glycoforms lacking the core fucose residue, and monoantennary glycoforms, such as A1G0F.

Typically, these CQAs would be measured using multiple analytical techniques, such as cation exchange liquid chromatography (charge variant profile), released glycan assays (glycans), peptide mapping, and liquid chromatography mass spectrometry (LC-MS) intact mass analysis (mass confirmation, intact glycoforms). Employing numerous techniques adds considerable time, complexity, and cost to the CQA monitoring process.[4] However, the ZipChip CVA-MS method provided a readout of valuable CQAs in a single, fast assay, replacing the use of many of these techniques for rapid product monitoring.

To evaluate the ability of the CVA-MS method to track CQAs over the course of a perfusion run, daily timepoints were prepared and analyzed. Time series analysis of samples taken throughout a perfusion CHO cell culture run (#179) showed that the main charge variant represented between 60% and 70%, while more acidic variants were typically in 25% to 30% abundance and the more basic species accounted for the remaining 5% to 10% (Figure 3A). This charge variant distribution was consistent with the mAb analyzed (undisclosed). Glycoform analysis showed that a higher relative abundance of more complex glycans was measured at the beginning of the cell culture run, while shorter glycan chains were detected later in the run (Figure 3B). While these glycoform changes were expected with this cell culture, the ability to track charge variants and glycoforms simultaneously provides greater insight into the process parameters impacting product quality.

The time series analysis also shows that charge variant and glycoform profiles in samples were consistent between the bioreactor and the permeate of the cell retention filter, providing assurance that the quality of the mAb harvested on the permeate side of the cell retention filter was not impacted by this filtration process.

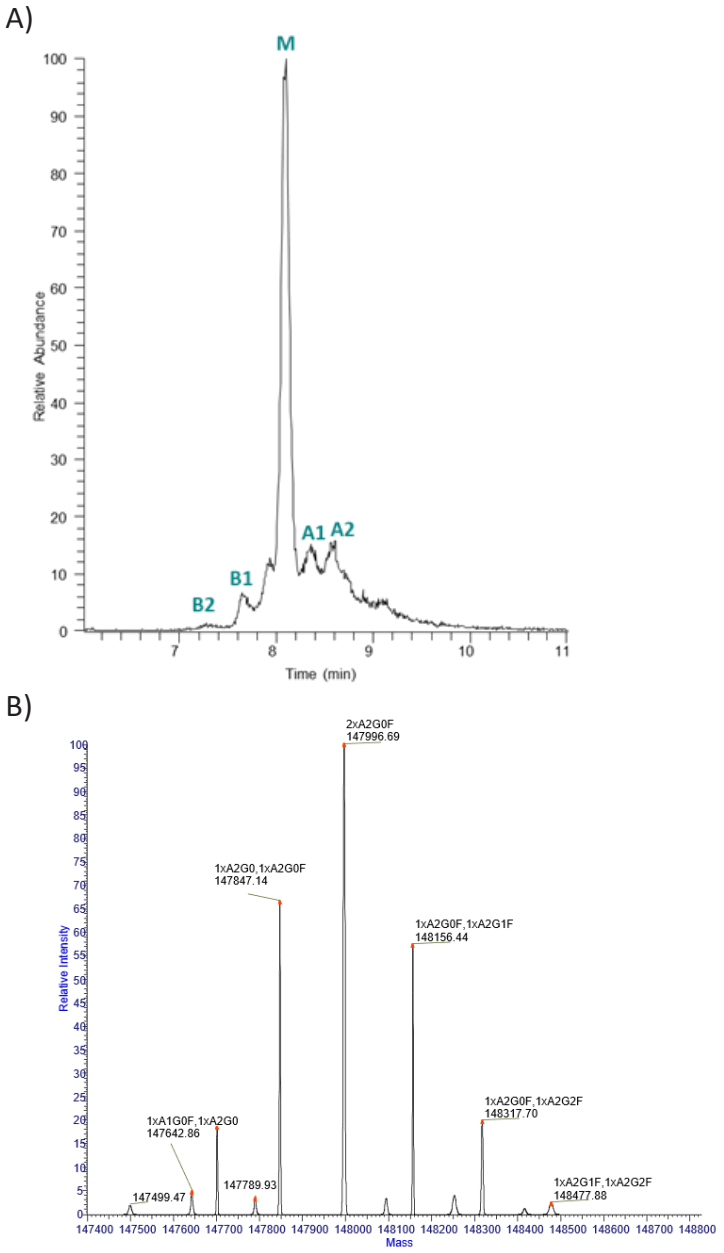

Figure 2. (A) example of a capillary zone electrophoresis elution profile from the ZipChip instrument, showing the five different charge variants detected; (B) example of a mass spectrum showing 12 well-resolved peaks and the corresponding glycoforms.

Finally, comparative analysis across multiple perfusion runs illustrates how the ZipChip platform can enable rapid identification of cell culture process variations to optimize mAb quality attributes (Figure 4). Cell cultures #179, #197, and #213 each used different perfusion processes throughout the course of the run. This resulted in different abundances of acidic and basic charge variants as well as glycoforms expressed in the mAb. This information can then be used to select or tune the bioprocess parameters to achieve the desired results.

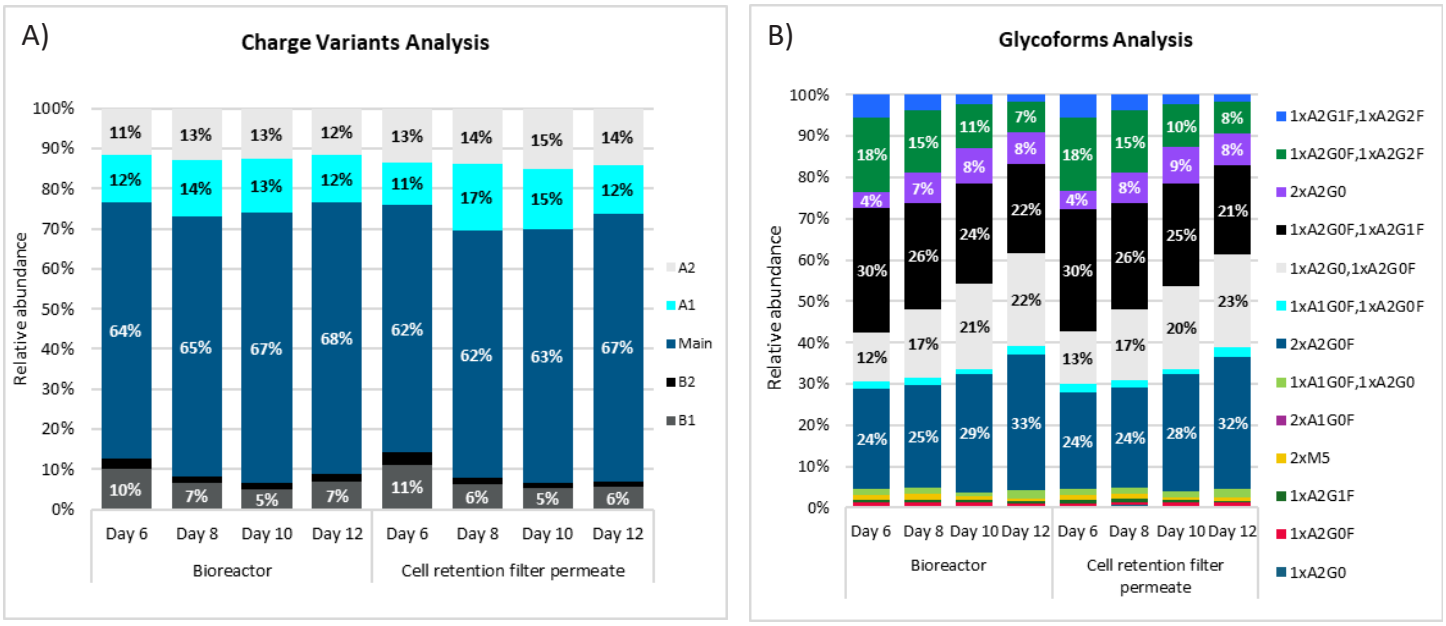

Figure 3. Time series analysis of charge variants (A) and glycoforms (B) in perfusion CHO cell culture samples from bioreactor and cell retention filter permeate. Samples from cell culture run #179 were taken on days 6, 8, 10, and 12 post-inoculation. The measured mAb titer ranged from ~0.4 g/L on day 6 to ~1 g/L on day 12.

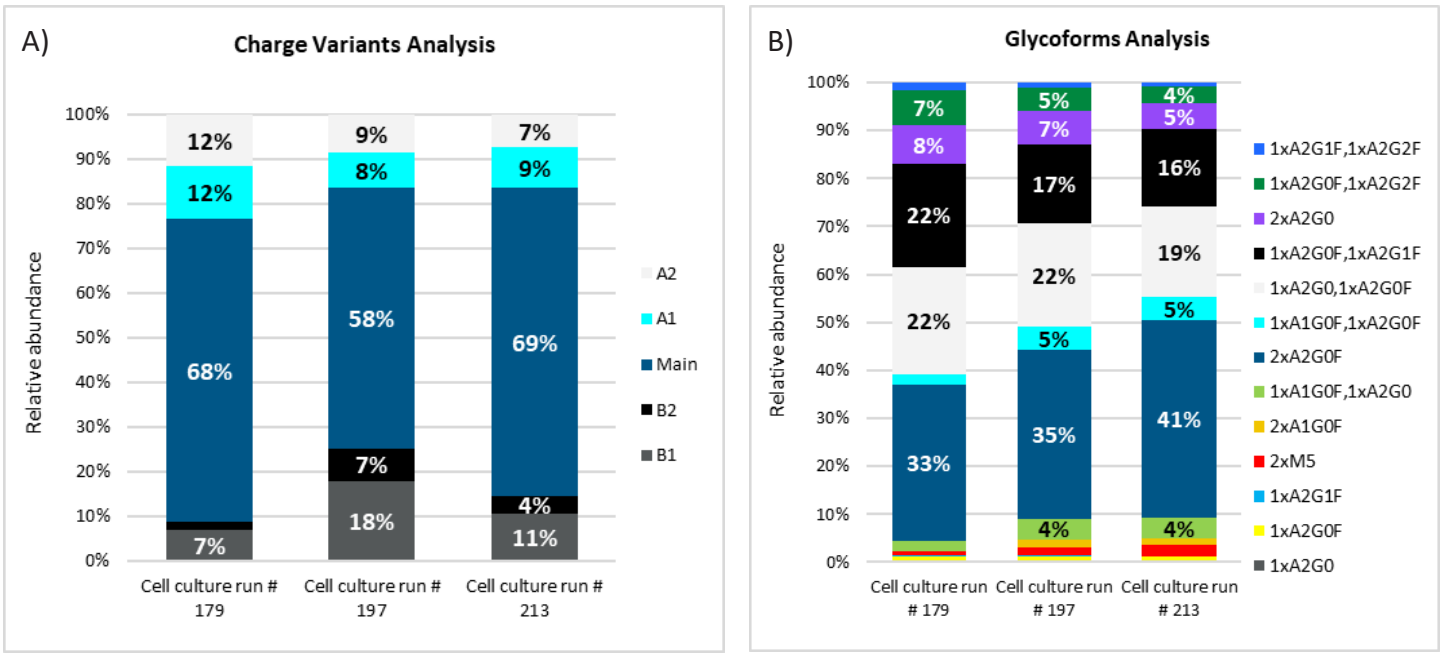

Figure 4. Comparative analysis of charge variants (A) and glycoforms (B) profiles from perfusion CHO cell culture runs #179, #197, and #213. Samples were taken from the bioreactor on day 12 post-inoculation.

Conclusion

This study demonstrates the utility of ZipChip-MS for direct monitoring of mAb quality attributes in perfusion CHO cultures. The ZipChip CVA-MS method monitored both charge variants and glycoforms of the expressed mAb at multiple timepoints of multiple perfusion cell cultures. Variations in charge variants and glycoforms across conditions were easily characterized and visualized. Detailed proteoform analysis confirmed that the quality attributes of the mAb were consistent between bioreactor and filtrate samples.

The ZipChip platform offers several key advantages for the analysis of mAb quality attributes directly from complex bioreactor media:

- **Minimal sample preparation:** Direct analysis of cell culture media eliminates lengthy prep workflows and complexity.
- **Multi-attribute readout:** Simultaneous monitoring of multiple CQAs from a simple, rapid method provides faster decision-making and improved process control.
- **High sensitivity:** Reliable detection at low titers enables insights into CQA trends sooner for earlier troubleshooting or intervention.

The simplicity of the method has the potential to enable real-time monitoring, which can facilitate reduced timelines for process development and rapid product release of commercial drugs. Collectively, these results establish ZipChip-MS as a powerful, high-throughput platform for monitoring mAb CQAs in perfusion processes, offering actionable insight into both bioreactor performance and harvest quality with a single, rapid assay.

References

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