Charge Variant Analysis of IgGs Directly from Cell Culture Supernatant using Microfluidic CE-ESI-MS

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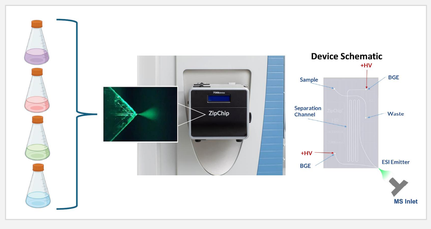
Introduction

As the field of bioprocessing continues to mature, there is a need for better analytics applied closer to the bioprocess. We have seen recent advancements in the technology used to measure small molecules within bioreactors (glucose/lactate, amino acids), but there is also a need to directly measure the critical quality attributes of the proteins being produced. To that end, we are assessing the ability of microfluidic CE-ESI-MS to perform charge variant - MS analysis of proteins pulled directly from a bioreactor. We know that excessive salt concentrations can cause band-broadening for this separation, so the key question is whether a desalting step is required, or if a simple dilute and shoot approach can work, and how much information can be obtained. Here we compare a simple dilute and shoot method to a method that buffer exchanges the sample before PATsmart™ ZipChip® analysis and demonstrates the use of this method for analysis of 4 different bioreactor growth conditions.

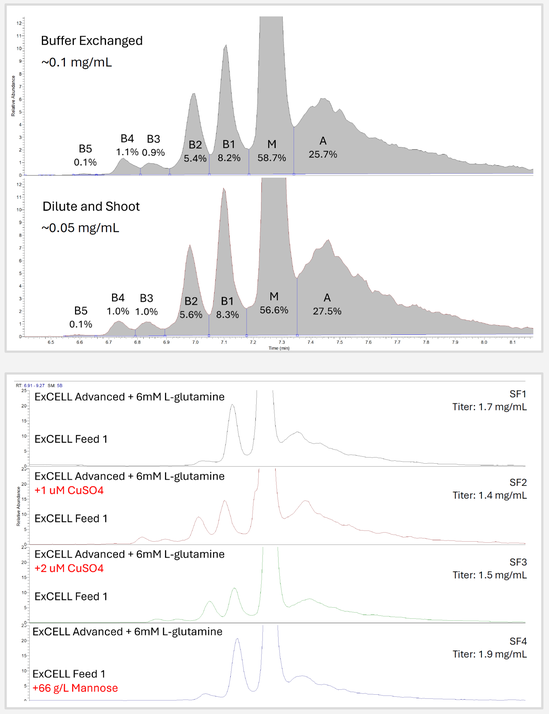
Materials and Methods

The NISTCHO cell line was cultured in shake flasks under four different growth conditions: standard conditions, two with elevated levels of copper sulfate, and one with elevated levels of mannose doped into the media. After 14 days of cell growth, aliquots of the media were collected from each shake flask and the expressed IgG was analyzed.

Charge variant analysis of the produced protein was performed using the ZipChip microfluidic CE-MS system (Repligen). An HRN chip and the Charge Variant Analysis kit (Repligen) were used for all analyses following the provided protocol. Data were acquired on an Exploris 240 Biopharma mass spectrometer (Thermo Fisher Scientific) and processed in BioPharma Finder 5.0 (Thermo Fisher Scientific) and UniDec v6.04.

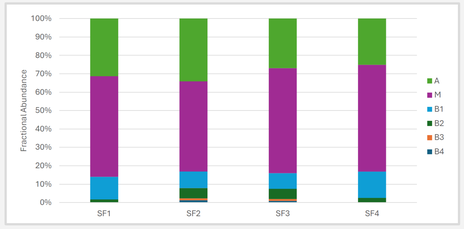


Results



Comparison of charge variant profiles obtained via buffer exchange and dilute and shoot workflows for SF3

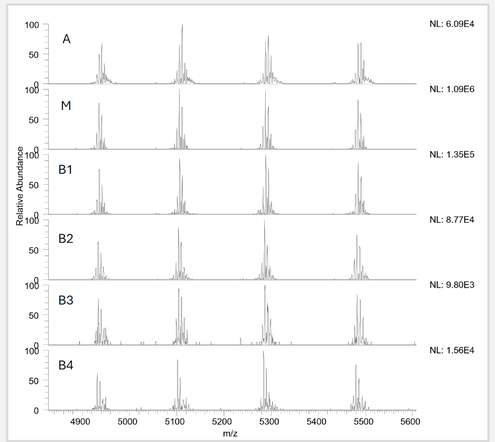
Charge variant profiles observed for all 4 shake flasks using the dilute and shoot workflow



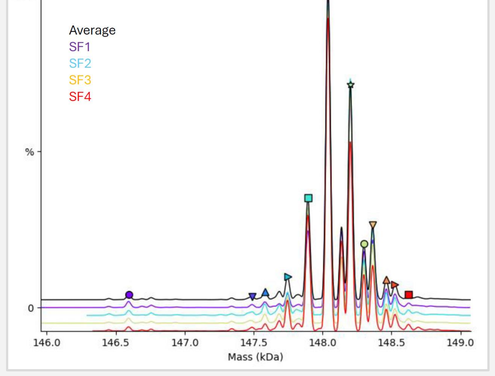
Comparison of charge variant peak areas observed from the 4 different shake flasks

	B4	B3	B2	B1	M	A
SF1			33.4 -2X pyroE	15.8 -1X pyroE	148040.6	178.7 Glycation + Oxidation
SF2	-117.9 2X Pro deamidation	-42.5 -1X pyroE + Pro deamidation	-58.8 Pro deamidation	17 -1X pyroE	148040.7	164.3 Glycation
SF3	-116.8 2X Pro deamidation	-42.5 -1X pyroE+ Pro deamidation	-58 Pro deamidation	16.2 -1X pyroE	148040.6	167 Glycation
SF4			31.8 -2X pyroE	17.4 -1X pyroE	148039.8	166.9 Glycation

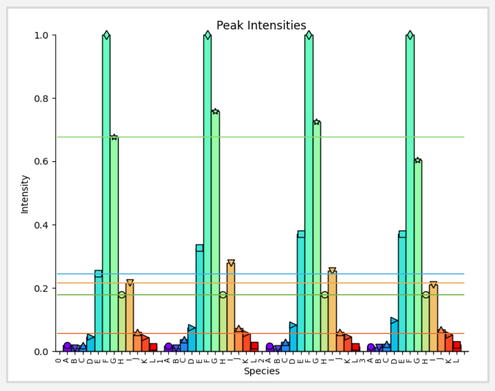
Identification of charge variants. Observed mass shifts and assignments are listed for each of the primary charge variant peaks from each bioreactor. The observed mass of the primary glycoform of the main peak is also listed for each.



Raw mass spectra of charge variants from the dilute and shoot method, zoomed to show the 4 most intense charge states. Spectral quality is sufficient to assign accurate masses for charge variants present at a fractional abundance of 1%.



The mass spectrum of the main charge variant peak from each shake flask was deconvolved using UniDec to facilitated a simple comparison of masses observed. These mass variant species correspond to glycosylation differences, with the primary glycoform identified as 2x A2G0F.



Relative abundances of the mass variants of the main charge variant observed from each of the 4 different growth conditions (SF1 – SF4 shown from left to right).

Discussion

These results demonstrate that the ZipChip CVA method works for mAbs pulled directly from a bioprocess vessel. The salts present in the growth media do not degrade the separation when samples are diluted at least 10x, and the sensitivity of this method yields high quality MS spectra for lower abundance variants even after significant dilution. The simple demonstration presented here illustrates how the charge variant profiles and mass variant comparisons could be used to gain critical insights into the performance of bioprocess runs. In this example, we compared 4 different growth conditions at the end of the bioprocess, but the same strategy could be used for real-time monitoring throughout the duration of a bioprocess.

Conclusions

- ZipChip charge variant – MS analysis works using simple dilute and shoot of cell culture supernatant
- Charge variant peaks can be identified using MS data at fractional abundance as low as 1%
- Significant differences in charge variant profiles can be seen in bioreactors with variable amounts of copper sulfate added
- Differences in the glycosylation pattern can be seen via simple deconvolution of the main peak observed from each sample
- The method demonstrated here could be used to monitor critical quality attributes in real time as a bioprocess is running.

References

UniDec software: Marty et al. Anal. Chem. 2015. DOI: 10.1021/acs.analchem.5b00140.

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