Supporting Bioprocessing of Monoclonal Antibody Based Therapies with PATsmart™ ZipChip® Microchip CE-MS

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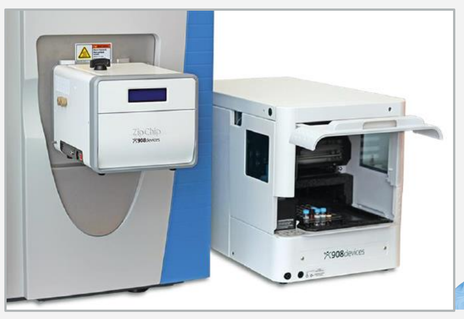
Overview

Manufacturing of biotherapeutic proteins relies on developing an expression system using live cell cultures to produce the drug product. Both the drug product and bioreactor environment must be monitored during upstream development and downstream manufacturing to ensure optimal growth and production conditions that lead to a consistent and high-quality product. Microchip CE-MS is a new technology which is uniquely well suited to characterizing both the small molecules in cell growth media, and the large proteins that are produced. By separating molecules using only an electric field in an open channel, it is possible for a single platform to achieve fast and efficient separations of molecules covering a wide range of molecular properties with virtually no sample prep. We demonstrate this unique capability here by measuring amino acids in growth media over a 14-day growth cycle; while using the same platform to measure accurate masses of intact mAb glycoforms.



Methods

Sample Preparation

Amino Acids in Growth Media

Bioreactor cultures of mammalian cells were grown using an Ambr ® 250 high throughput system Ambr Bioreactor System, Sartorius). Three separate cell cultures were used to generate this data. Ammonia was added 12 hours post inoculation to two of the bioreactor vessels to stress the cultures during the growth cycle, while one vessel was left unperturbed to represent more standard growth conditions (0 mM, 10 mM, and 30 mM ammonia added).

Samples were derived from time points taken over the course of a 14-day growth cycle. The collected spent media time points were stored at 20°C until analysis. Spent media samples were first diluted 1000-fold with LC/MS grade water to bring the concentration of media components into the linear dynamic range of the system. The diluted samples were then diluted an additional 10-fold with 80/20 water/methanol containing 100 mM ammonium acetate and 1 µM stable isotope labeled amino acids to serve as internal standards (Cambridge Isotope Laboratories, Inc.).

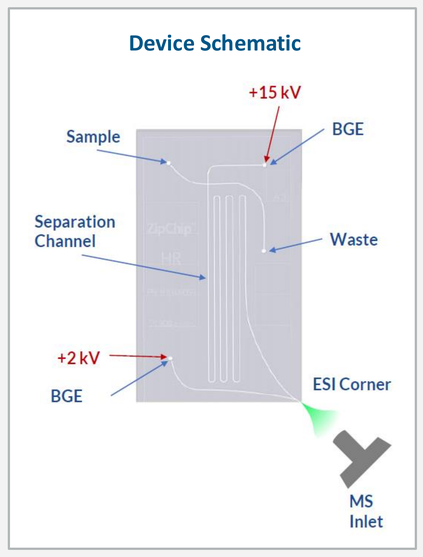
For quantitation, a calibration curve was prepared containing the 20 essential amino acids. The curve was created using 4 points at 0.5 µM, 5 µM, 50 µM, and 500 µM which were then diluted 10-fold with the solution containing internal standards, for a final concentration of 0.05 µM, 0.5 µM, 5 µM, and 50 µM.

Intact mAb Analysis

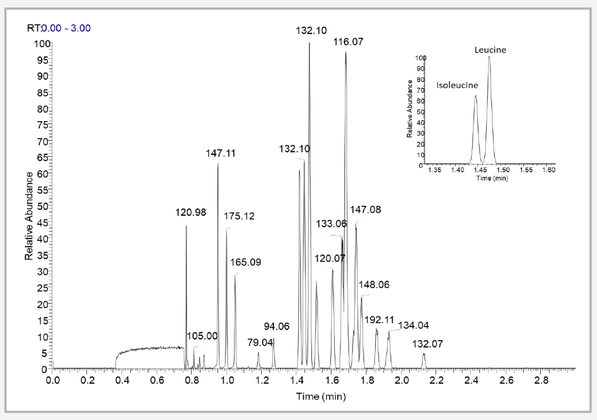
mAb samples were diluted with background electrolyte to a concentration of 0.5 mg/mL.

Instrumentation

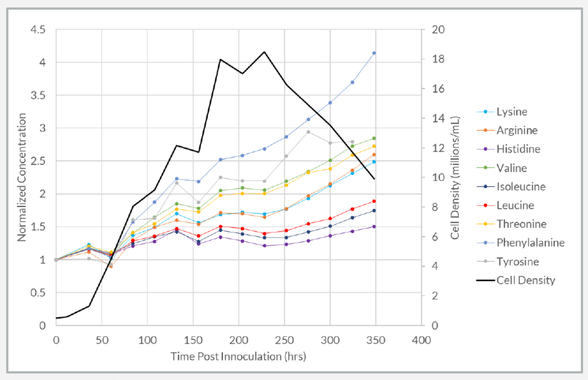
The PATsmart™ ZipChip® microchip CE-MS system (Repligen) attached to an Exactive Plus EMR, orbitrap mass spectrometer (Thermo Fisher Scientific) was used for this work. The microfluidic device design, as seen to the right, incorporates an injection cross, serpentine separation channel, and an integrated ESI emitter where electrospray is generated directly off the corner of the device. Microfluidic chips with a 10 cm long separation channel (HS, Repligen) were used for both assays described here.

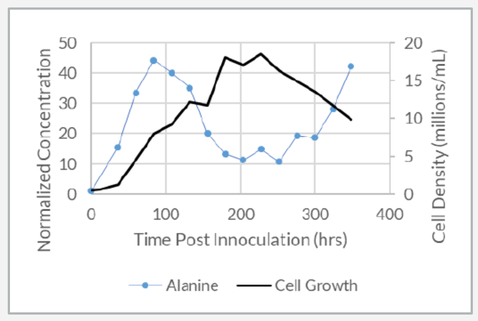


Amino Acid Quantitation from Bioreactors

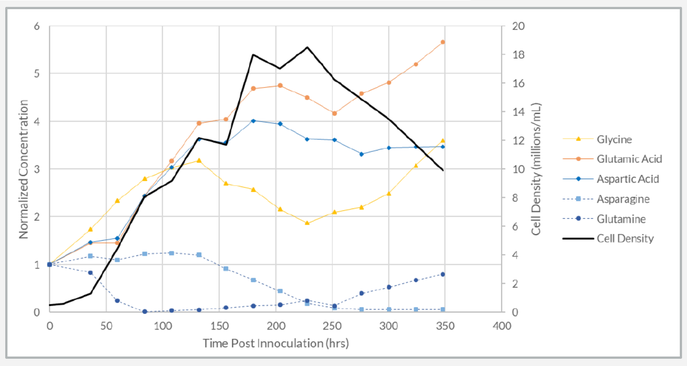


Analyte	m/z	Migration Time (min)	Analyte	m/z	Migration Time (min)
Lysine	147.11	0.95	Threonine	120.07	1.61
Lysine heavy	155.13	0.95	Threonine heavy	125.08	1.61
Arginine	175.12	0.99	Methionine	150.06	1.61
Arginine heavy	185.13	0.99	Methionine heavy	156.07	1.61
Histidine	156.08	1.05	Asparagine	133.06	1.66
Histidine heavy	165.09	1.05	Proline	116.07	1.68
Glycine	76.04	1.18	Proline heavy	122.08	1.68
Glycine heavy	79.04	1.18	Phenylalanine	166.09	1.69
Alanine	90.06	1.27	Phenylalanine heavy	176.11	1.69
Alanine heavy	94.06	1.27	Tryptophan	205.10	1.73
Valine	118.09	1.42	Glutamine	147.08	1.74
Valine heavy	124.10	1.42	Glutamic Acid	148.06	1.77
Isoleucine	132.10	1.44	Glutamic Acid heavy	154.07	1.77
Isoleucine heavy	139.12	1.44	Tyrosine	182.08	1.86
Leucine	132.10	1.48	Tyrosine heavy	192.11	1.86
Leucine heavy	139.12	1.48	Aspartic Acid	134.04	1.93
Serine	106.05	1.52	Aspartic Acid heavy	139.06	1.93
Serine heavy	110.06	1.61			

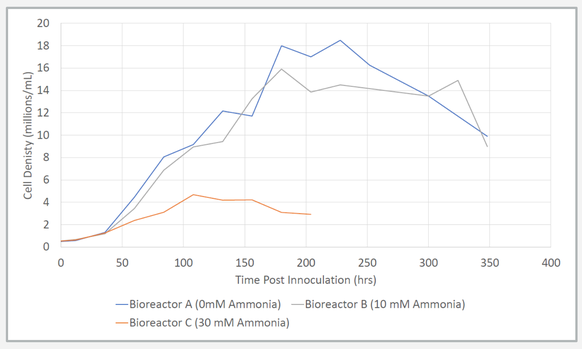


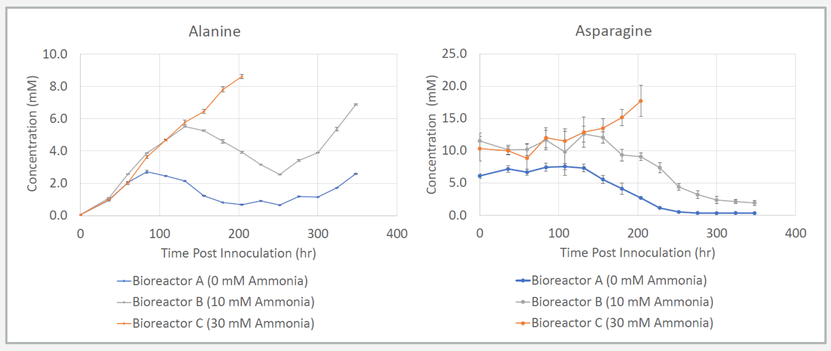


ZipChip separation of the t0 timepoint from Bioreactor A. The amino acids migrate through the chip in less than 3 minutes. As shown in the inset, isomers such as isoleucine and leucine are resolved and detected as separate peaks for confident identification and quantitation.



The above plots show amino acid concentrations versus cell growth time for the healthy bioreactor. We see that many of the amino acids increase in concentration over time as they are being continuously added to the bioreactor in the feed mix. Some of the amino acids decrease in concentration as the cell density increases, indicating that the cells are consuming these amino acids.



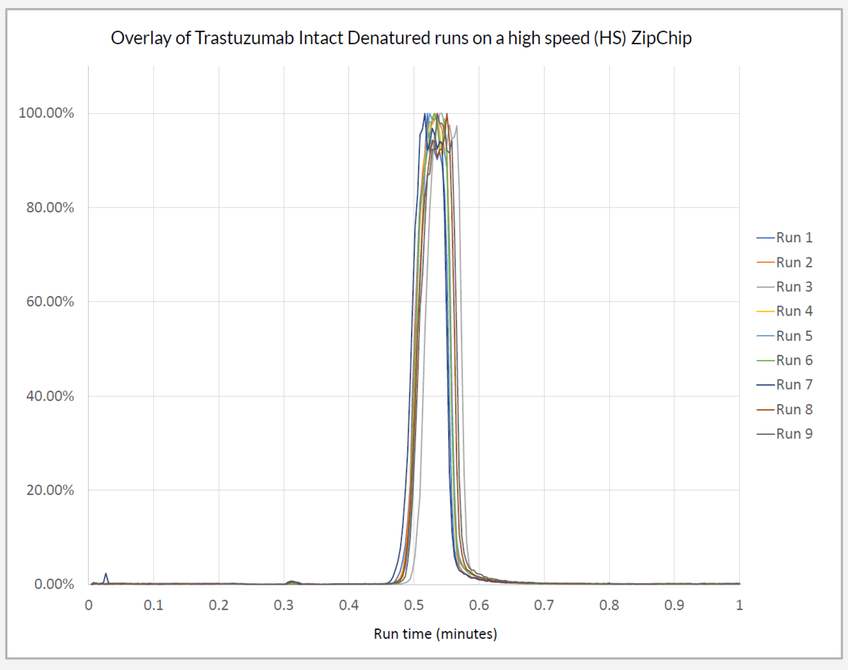


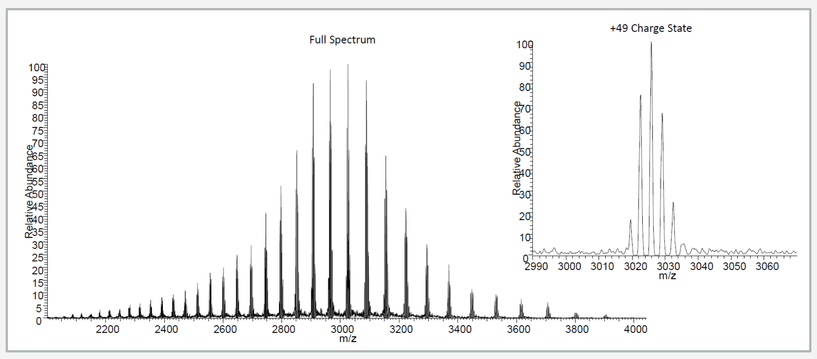
This feedback could be used to optimized the feed mixtures in real time. The plots at left indicate how the stressed bioreactors display clear differences in amino acid consumption rate. The specific examples of alanine and asparagine show that monitoring the amino acids can indicate a problem in the bioreactor before differences in cell density become evident.

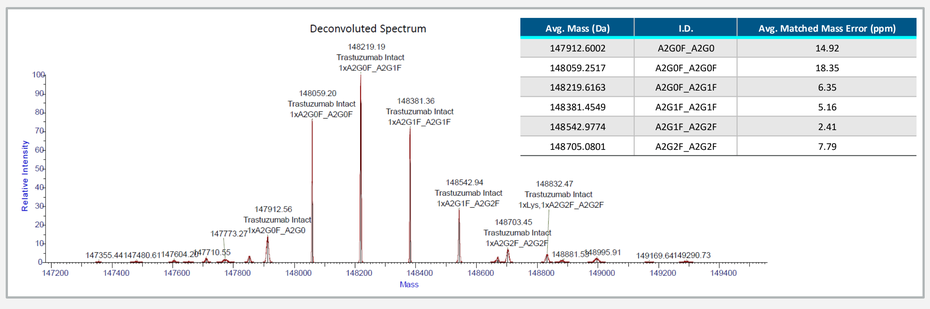
Intact mAb Characterization

The ZipChip electrophoretic separation rapidly desalts the mAb and delivers a clean band of antibody to the MS in only 30 seconds. This high throughput method offers a simple and robust workflow for accurate intact mass analysis of drug products. Because this method delivers the mAb in a single analyte band, data processing is extremely simple. The MS signal is averaged across the full peak width to generate a full spectrum like that shown at right.

This spectrum can then be deconvoluted to yield accurate masses for the primary glycoforms and mass variant peaks observed. Additional ZipChip methods can be run under native conditions to separate charge variants for a more detailed characterization.







Avg. Mass (Da)	I.D.	Avg. Matched Mass Error (ppm)
147912.6002	A2G0F_A2G0	14.92
148059.2517	A2G0F_A2G0F	18.35
148219.6163	A2G0F_A2G1F	6.35
148381.4549	A2G1F_A2G1F	5.16
148542.9774	A2G1F_A2G2F	2.41
148705.0801	A2G2F_A2G2F	7.79

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