An Automated Novel On-Line Sample Processing Methodology Using Mass Spectrometry to Determine Bioreactor Nutrient Levels and Real-Time Product Characterization

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Abstract

Bioreactor parameters can have significant effects on the quantity and quality of biotherapeutics. With mAb products, one particularly important CQA is the distribution of product glycoforms. N-linked glycosylation affects the therapeutic properties of the antibody, including effector function, immunogenicity, stability, and clearance rate. The conditions under which the biotherapeutic product was produced can have a significant impact on the drug's quality. To facilitate real-time analysis of bioreactor parameters and the glycosylation state of the antibody products, we have developed a novel on-line system to pull cell-free samples directly from the bioreactors, chemically process them, and deliver them to a chromatography mass spectroscopy system for identification and quantification. This system can currently identify and quantitate amino acid levels in bioreactor media within about twenty minutes after pulling a sample from the bioreactors and can be programmed to automatically pull and process samples. Our work involving nutrient effects on product quality has revealed how different media and individual amino acids may affect the final glycosylation state. For example, using different amino acid blends for supplementation can affect the cell growth and final glycan profile of the antibody product. By understanding how bioreactor nutrients can affect product quality, out-of-specification product batches could be avoided by careful control of the media parameters.

Introduction

Monoclonal antibodies (mAbs) constitute a majority of the biotherapeutic market and are a class of drugs that will continue to expand due the rapid development of emerging biosimilars with CHO cell manufacturing being the predominant platform. Biotherapeutics are produced within living systems, which results in intrinsic protein product variability due to the complex nature of bioreactor cell cultures. To overcome batch-to-batch variability and achieve consistent high-quality product, key process parameters that affect the product efficacy must be closely monitored. Process analytical technology (PAT) is a mechanism designed to oversee and control a manufacturing process to ensure reproducible, high-quality product through measurement of critical process parameters (CPP) which affect product quality attributes (PQA). To this end, PAT offers great potential for process development such as root cause analysis which can improve process understanding with the data collected in multiple batches by granting further insight into the factors that generate variability in product quality.

We are interested in automated PAT for monitoring both bioreactor nutrient conditions and product quality. Spectroscopic methods are powerful and widely used PAT techniques implemented as real-time process controls, and it has been demonstrated that in-line Raman spectroscopy PAT can monitor real-time amino acid concentrations in cell culture process. However, spectroscopy signals can be limited in selectivity when analyzing several components such as amino acids within a complex culture broth. On-line high-performance liquid chromatography (HPLC) can overcome these limitations with its ability to utilize a robust method to provide fast analytical output. However, the application of these techniques in a process stream as on-line PAT is a challenge as it is a relatively complex technology. In our study, we demonstrate real-time on-line monitoring via HPLC to assess amino acid concentrations in various cell culture feeding methods while comparing these effects on the glycan profile of a model IgG1 mAb.

Materials and Methods

A Seg-Flow 4800 (Flownamics) pulled cell-free samples from bioreactors with a FISP probe with a 0.2 µm filter: one cycle provided one sample every four hours from three processing units, and sample lines were cleaned with 70% isopropanol daily. Sample lines were drawn to a ProSIA (FIAlab) for amino acid derivatization using AccQTag kit reagents (Waters). A vial of each reagent was directly attached to the ProSIA: AccQTag Borate Buffer, and Ultrareagent Powder reconstituted in acetonitrile. For amino acid derivatization, cell-free media was mixed with AccQTag Ultra Borate Buffer and AccQTag reagent for 120 secs, then held for 1 minute at room temperature. The mixture was sent to a heat block set to 55°C and held for 10 mins to terminate the reaction before the derivatized sample was sent to the Patrol UPLC with a single quadrupole mass detection.
An AccQ-Tag Ultra RP column, 100 x 2.1 mm, 1.7 um (Waters) was used for separations. Between sample runs the column was held at a lowered flow rate, then ramped up flow rate before sample injection for proper equilibration. AccQTag Amino Acid standards (Waters) were run in serial dilution as a calibration curve performed before the processing units were inoculated and after all processing units were harvested, and an amino acid standard was run daily. All standard and sample chromatographs were processed with Empower3 (Waters). Figure 1 illustrates the experiment set up for real-time amino acid analysis. All instrument communication was integrated as an OPC network with Python to program sampling cycles, ProSIA's amino acid derivatization steps, and method start on Patrol UPLC system.

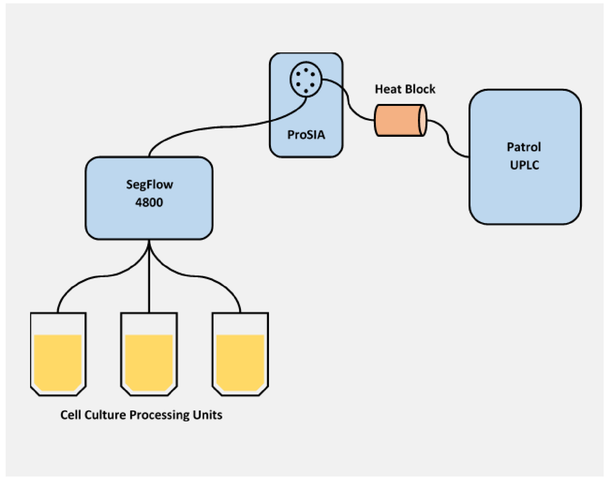

Figure 1. Illustration of the OPC network connections integrated with Python. Cell-free culture broth was pulled from processing units with the SegFlow 4800 and sent to the ProSIA for automated amino acid derivatization. After the derivatized sample was heated in the heat block, the sample was injected to a Patrol UPLC analysis system with a single quadrupole mass spectrometry detector. All instruments were coordinated in synced sampling cycles via Python scripts run by the ProSIA system.

Results and Discussion

We conducted two sets of bioreactor runs: the first was focused on amino acid supplementation strategies without automated analysis, while the second was conducted to test the online amino acid quantification PAT system we developed.
Table 1 outlines the feed strategies used for the amino acid supplementation study, where amino acid blends were added to bioreactors instead of media feeds. These feeds caused different VCD responses in the bioreactors (Figure 2), where the blend of Tyr, Cys, Pro and Asn had a marked effect on VCD rebound while a blend of Met, His, Trp and Thr did not. These amino acid blends also caused differences in the glycan profile of the produced mAb product, as shown in Figure 3.
To facilitate additional study into amino acid effects on bioreactor productivity, we created an online amino acid quantification system as shown in Figure 1. We tested this system with three bioreactors, and the real-time measurements for proline are shown in Figure 4.

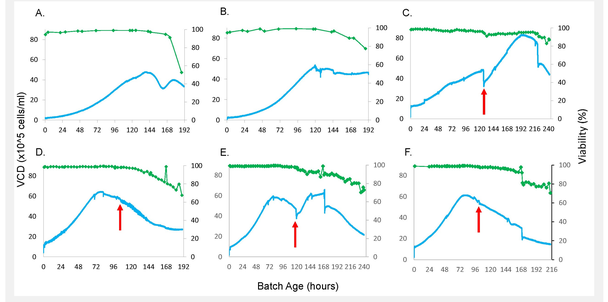

Figure 2. Growth profiles for different feeding strategies. VCD (10^5 cells/mL) is shown in blue as measured by bio-capacitance and green data points are viability (%) measurements. The red arrows indicate when the first amino acid supplementation event occurred. (A) Batch strategy 1 (B) Fed-batch strategy 2 (C) Fed-batch and amino acid supplementation strategy 4 (D) Fed-batch and amino acid supplementation strategy 5 (E) Fed-batch and amino acid supplementation strategy 6 (F) Fed-batch and amino acid supplementation strategy 7.

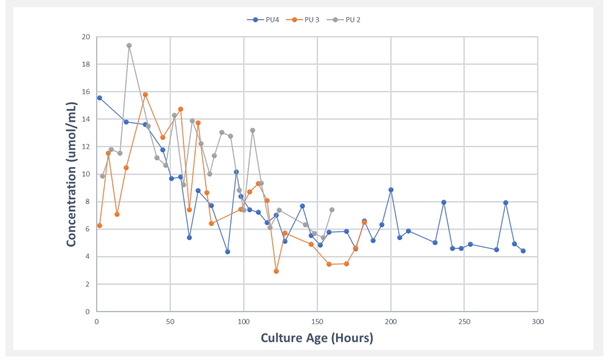

Figure 4. Representative online real-time quantification results for proline from three bioreactors run in three different feeding strategies.

Table 1. Feeding and amino acid supplementation strategies for nine bioreactors used in amino acid supplementation study.

Feed strategy	Number of bioreactors	Glucose feed initiation (g/L)	Glucose feed added (g/L)	Glutamine feed initiation (mM)	Glutamine feed added (mM)	Amino acid blends (first, second)*	Amino acid addition time (hour)
1	1	--	--	--	--	--	--
2	2	<1.5	1	1	2	--	--
3	2	<0.2	6	<1	8	--	--
4	1	<2.5	2.5	4	4	A, A+B	127, 221
5	1	<1	7	1	9	B, C	105, 166
6	1	<1	7	1	9	A, A+B	119, 170
7	1	<2.5	2.5	4	4	B, D	77, 170

Amino acid blend A: Tyr, Cys, Pro, Asn
Amino acid blend B: Met, His, Trp, Thr
Amino acid blend C: Cys, Asn
Amino acid blend D: Tyr, Pro

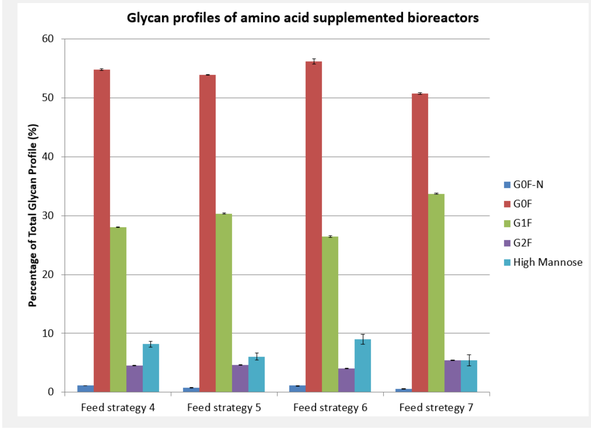

Figure 3. Final glycan profiles of mAb products. The final mAb products from the four bioreactor cultures supplemented with amino acids were characterized for the glycan profile. Feed strategies 4 and 6 both started with amino acid blend A, while feed strategies 5 and 7 used amino acid blend B.

Conclusions

- Concentrations of amino acids in the bioreactor media may affect product quality outcomes.
- Automated monitoring and regulation of nutrient levels in the bioreactor may help ensure product quality.
- In this study, we demonstrate amino acid quantification strategies and how their levels may affect the final glycan profiles of protein products.
- Our online system is currently able to quantitate amino acid concentrations from spent bioreactor media, and we are working on upgrading the system to be able to perform cleaved glycan analysis for assessing the antibody glycan profile in near real-time.

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