Advancing understanding of bioreactor and process parameter impacts on antibody product quality outcomes through the development of mass spectrometry-based process analytical technology

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Abstract

Bioprocessing is a complex process through which engineered cells are used as manufacturing plants for generating complex protein biotherapeutics. As living cells, changes in bioreactor conditions and process parameters can have unexpected impacts to their growth parameters and thus, product quality. To address this issue and maintain product quality through process optimization, we have worked with advances in rapid analytical methodologies to characterize bioreactor media parameters and product quality attributes (PQA) in speeds approaching real time. These process analytical technology (PAT) approaches have included complex on-line systems and streamlined at-line instrumentation. Here, we present our ongoing work on predominately mass spectrometry-based instrumentation used to assess bioreactor media metabolites and nutrients, as well as evaluate product quality attributes such as the glycosylation profile of monoclonal antibody (mAb) protein products in less than an hour. At-line instruments tend to be stand-alone "black box" modules with refined user interfaces and ease-of-use, but at high-cost and little user flexibility (i.e. the analytes/parameters that can be studied are predetermined and use expensive pre-packaged kits). On the other hand, current efforts in on-line systems are generally still early in developmental stages due to difficulties in interfacing equipment from different companies. These challenges include both physically connecting the equipment together and getting them to communicate to facilitate a complete analytical platform. Our group has worked with both at-line and on-line approaches, and understand their pros and cons. This will be described along with our continuing work in coupling bioreactor process parameters and product quality outcomes in mAb bioproduction using statistical approaches such as multi-variate data analysis (MVDA).

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.

Materials and Methods

For online amino acid analysis, a Seg-Flow 4800 (Flownamics) pulled cell-free media 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 below 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.

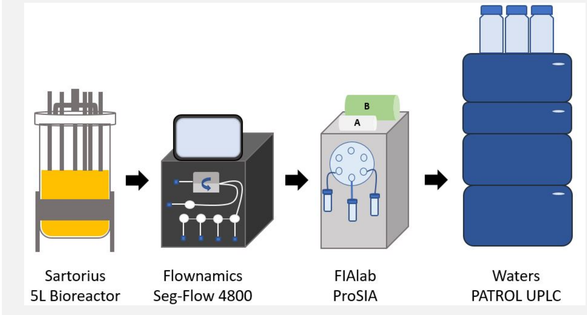

Figure 1. Illustration of the online amino acid analysis platform. Cell-free culture broth was pulled from the bioreactor with the SegFlow 4800 and sent to the ProSIA for automated amino acid derivatization. After the derivatized sample mixed in a coil (A), and heated in the heat block (B), the sample 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.

Figure 3. The 908devices REBEL at-line cell culture media analyzer which can provide results on 30+ components in under 10 minutes. The analytes that can be quantified include amino acids, biogenic amines, and vitamins.

Glycan samples were run at-line by utilizing a FIAlab ProSIA setup, which can isolate and label glycans using Agilent Instant PC (IPC) reagents. Work is in progress to make the glycan analysis online. We collaborated with researchers at Rutgers University who had developed an offline glycan characterization technology, dubbed the N-GLYcanyzer. At FDA, the N-GLYcanyzer was adapted to provide rapid at-line determination of the glycan profile for a perfusion bioreactor experiment. The N-GLYcanyzer uses the InstantPC glycan analysis workflow developed by Agilent Technologies. A schematic of the N-GLYcanyzer automated method can be found below in Figure 2.

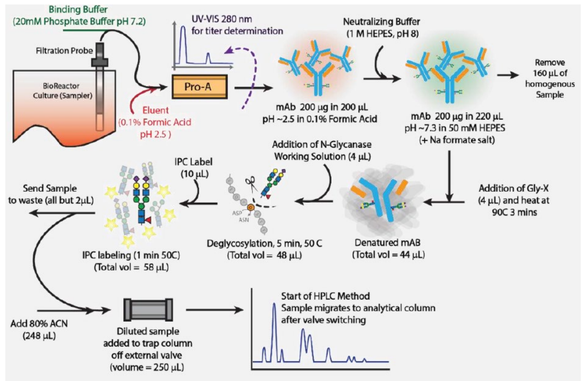

Figure 2. Illustration of the at-line glycan analysis platform, the N-GLYcanyzer. Briefly, cell-free media samples are manually pulled from the bioreactor and passed to the N-GLYcanyzer setup, which starts at the Pro-A cleanup of mAb. After the mAb is isolated on the Pro-A column, it is denatured with the glycans cleaved using N-Glycanase and labeled with IPC. A trapping column is then used to purify the labeled glycans from the solution so these can be passed onto an HPLC for analysis.

In addition to our efforts to develop online analytical capabilities for rapid bioreactor metabolomic characterization and product quality determination, we are assessing the use of rapid at-line instrumentation that has recently been marketed to allow for swift results without needing a background in advanced instrumentation such as a mass spectrometer or HPLC. An example of one such device is the REBEL Cell Culture Analyzer from 908devices, which combines capillary electrophoresis and high-pressure mass spectrometry in a user-friendly presentation (Figure 3, left).

We are currently comparing the absolute concentration determination of the REBEL against label-free HPLC mass spectrometry approaches. Each approach has its own advantages and disadvantages. Once this work is completed, we will be publishing our findings.

Results and Discussion

			Media			
Analytical Method	Glycan Type (%)	Glycan Structure	Ex-Cell Advanced	OptiCHO	PowerCHO2	ProCHO5
Mass Spectrometry	G0F		39.618 ± 4.100	**53.189 ± 5.041**	46.314 ± 4.054	36.212 ± 2.752
	G1F		33.016 ± 1.324	28.269 ± 1.37	32.58 ± 5.775	**43.305 ± 1.549**
	G2F		6.573 ± 0.720	4.154 ± 0.625	6.069 ± 2.384	**11.044 ± 1.072**
	High Mannose		**16.149 ± 2.609**	10.250 ± 2.586	10.982 ± 1.069	5.539 ± 2.071
	Other		4.625 ± 1.915	4.139 ± 1.201	4.054 ± 2.751	3.900 ± 1.288
rCE-SDS	Aglycosylation		4.342 ± 2.450	4.514 ± 0.807	**8.924 ± 2.736**	3.511 ± 2.059

Table 1. Glycosylation profiles by medium. The numbers represent the percentage of the total glycan profile for all labeled glycan data obtained by mass spectrometry which sum to 100%. The rCE-SDS aglycosylation values represent the percentage of antibody heavy chains are not glycosylated versus those that are. The error values indicated are standard deviation values for all biological replicates and technical replicates measured. The bold values are the highest values for each glycan type.

Our results have indicated that the nutrient environment of the bioreactor can have profound impacts on the product quality outcomes of the produced antibody. For example, when we cultured the same cell line producing the same mAb product in different media, each media produced a different glycan profile (Table 1, above). Ex-Cell Advanced media produced more high mannose glycoforms than ProCHO5 which produced the most galactosylated glycoforms (G1F and G2F). Additionally, when we have used unique amino acid supplementation during a bioreactor run we have found some amino acids can prolong the culture life while others do not, with this also impacting the glycan profile.

To better understand how the nutrient environment impacts the product quality outcomes of antibody therapeutics, we are actively developing online analytical capabilities that could be used for rapid corrective actions during the culture, especially considering the current push towards continuous manufacturing.

Conclusions

- Concentrations of amino acids in the bioreactor media may affect product quality outcomes, such as the glycan profile.
- 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 an online fashion.

References: A. Gyorgypal et al, *Reaction Chemistry & Engineering*, under review; N. Azer et al, *Biotechnol Prog*, under review; D. Powers et al, *Biotechnol Prog*, 2019.

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