Raman spectrometry as a tool to study minimization of batch age effects and make product quality decisions for biotherapeutic antibody production

Kohnhorst, Casey L.[1], Azer, Nicole[1], Bush, Xin[1,2], Trunfio, Nicholas[1], Berilla, Erica[1] , Powers, David N.[1]

1: Food and Drug Administration, Office of Pharmaceutical Quality, Office of Biotechnology Products, Division of Biotechnology Revision and Review II, Silver Spring, Maryland.

2: Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, Rhode Island.

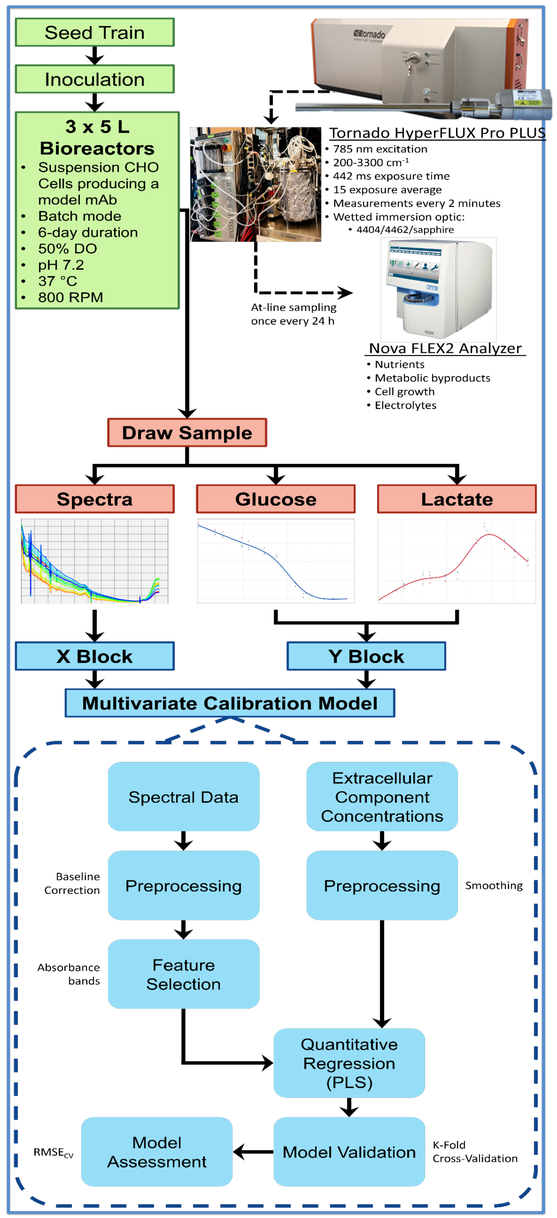
Abstract

During upstream manufacturing of biotechnology products, subtle changes in the media and in-process parameters can result in significant changes to protein structure and post-translational modifications, such as glycosylation, generating clinically relevant differences. These uncharacterized effects on product quality could go unnoticed during manufacturing as product characterization often does not occur until days to weeks after the harvest is complete since the product must be shipped to a characterization lab for analysis. Due to this time gap, development of real-time process analytical technology (PAT) is of great value as real-time monitoring, feed-back, and control could have allowed the batch to be rescued. Raman spectroscopy can nondestructively assay chemical composition and molecular structure using visible or near-infrared radiation, making it an ideal PAT to monitor bioreactor conditions in real-time. However, as Raman spectra are complex this precludes the use of univariate quantification methods that calculate the various components' concentration from specific peaks. Thus, multivariate calibration models are used to characterize upstream bioprocesses from spectroscopic measurements. Ideally, a statistically rigorous design of experiment would be employed to generate a robust dataset for model training. However, cellular metabolism ensures that nutrient and byproduct concentrations will vary collinearly with one another – the "batch age effect". Being unable to change the various components' concentration independently of one another results in a training data set that has inadequate design space coverage, leading to spurious soft sensor models whose artificially good performance is lost when the components' concentration collinearity differs from the training set. This study will show that commonly used model summary statistics can overstate the reproducibility of a given multivariate calibration model trained from data generated during upstream biomanufacturing processes. We will also demonstrate that the degree to which a given model's performance is dependent on that collinearity can be assessed from its score space and that supplementation experiments can be used to decrease model dependence on component collinearity.

Introduction

- Nutrient depletion and byproduct accumulation in a bioreactor can cause undesirable effects on therapeutic proteins' structure and post-translational modifications, potentially impacting product quality and clinical efficacy and safety. Currently, the nutrient and metabolite data are measured at-line or off-line 1 to 3 times a day. This infrequent measurement can mean inadequate monitoring and control of the bioreactor.
- Raman spectroscopy is a promising tool for real-time, continuous process monitoring during the upstream manufacturing of biotherapeutics.
- However, Raman spectra are extremely complex. The numerous extracellular chemical species present in a cell culture have similar spectral absorbance characteristics which preclude the use of univariate quantification methods that calculate the various components' concentrations from specific peaks. Thus, multivariate calibration models should be used to characterize the bioreactor cell culture from spectroscopic measurements.
- Ideally, a statistically rigorous Design of Experiment (DoE) would be used to generate a robust dataset for model training, accounting for aliasing between the various components' absorbance characteristics. However, cellular metabolism ensures that nutrient and byproduct concentrations will vary collinearly with one another. For example, glucose consumption leads to lactate production. This is known as the "Batch Age Effect."
- The Batch Age Effect can lead to spurious soft sensor models whose artificially good performance is lost when the components' concentration collinearity differs from the training set.
- To address this, we will use byproduct bolus additions to break the collinearity between batch age and accumulation of these analytes to better allow our MVDA models to associate true changes in bioreactor parameters and product quality changes to byproduct changes and not to the confounding association to batch age.
- **To date, we are currently working on the development of Raman spectroscopy-based predictive models to monitor glucose and lactate within our bioreactors for a Chinese Hamster Ovary (CHO) cell line producing a model monoclonal antibody (mAb).**

Materials and Methods



Results and Discussion

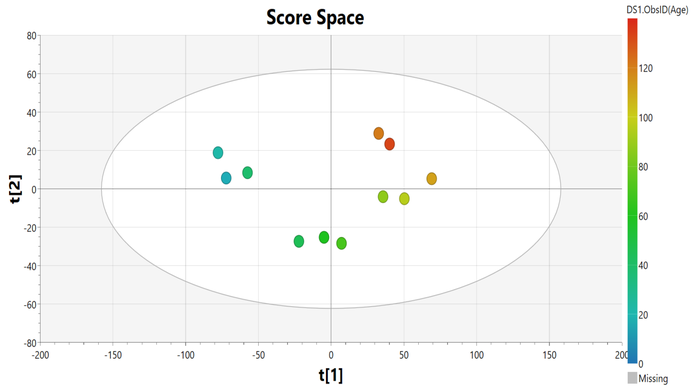


Figure 1. Score space for the PLS model of preprocessed spectra and glucose concentrations. Color gradient indicates the batch age in hours.

- Initially, the PLS model extracted 2 principal components (Fig.1), where the number to extract was determined by 7-fold cross-validation.
- Score 1, which accounts for more than 80% of the variability in the feature space, varies roughly linearly with time.
- As PLS models are expressed as linear functions of the scores, any chemical species that varies linearly with time will be predicted from the net effect on the spectra from all chemical species that vary linearly with time.
- R^2 and Q^2 were calculated to help assess model performance (Table 1). R^2 Represents the fraction of variance in the spectra (X) and the reactor variables, such as [glucose] (Y). Q^2 Represents the ability of the model to predict bioreactor variables from the Raman spectra; the predictable fraction of data captured by the model.

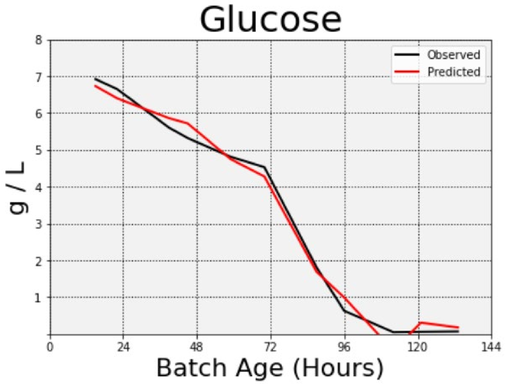


Figure 2. Model predicted glucose values vs. observed.

Table 1. PLS model summary statistics.

	$R^2(X)$	$R^2(Y)$	Q^2
PC1	0.859	0.833	0.793
PC2	0.991	0.898	0.850

- The model summary statistics (Table 1) indicate that more than 75% of the variability seen in the spectra (X), and in the glucose concentration (Y), was captured by the model.
- Model prediction accuracy is good; as seen from the low prediction error in the glucose concentrations in the observed vs. predicted profiles (Fig. 2).
- The cross-validated Q^2 value indicates that the results should be reproducible.

Conclusion

- The degree to which a given model's performance is dependent on component concentration collinearity can be assessed from its score space's temporal trajectory. This effect was particularly evident for Score 1.
- It has been shown here that model summary statistics – such as R2, Q2 and prediction error – can overstate the reproducibility of a given multivariate calibration model even when that model is confounded by batch age effects.
- Work is ongoing to further develop this glucose model, as well as one for lactate. Once the models are prepared, we will utilize timed bolus additions to break this collinearity between the response parameters and time. Breaking the collinearity is expected to improve the reproducibility of the models' predictions.