Strategic Developing Process Analytical Technologies for Real Time Quality Attribute Analysis in Bioprocess Development

November 2021

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Biologics Development

Bristol Myers Squibb
BMS Biologics Development Network

PROCESS/ANALYTICAL DEVELOPMENT & CLINICAL MANUFACTURING

Devens, MA
- US Biologics COE
- Biologic Development
- MS&T, Quality AS&T
- Clinical Manufacturing
- Commercial Manufacturing

New Brunswick, NJ
US Biologic Development - Early Phase
Bioassay COE; GMP Testing

Summit, NJ
US Biologic Development - Early Phase
Clinical Manufacturing
Outline

• Strategic Developing our Technology Roadmap Towards Real Time Product Quality and Bioprocess Monitoring

• PAT Vision and Technology Landscape at BMS with Case Studies

• What will this lead us into the future? - Future Outlook
Traditional Process-Analytical Integration vs. PAT

Modes of Integration

- **In-line**
  - Samples are analyzed within the process stream

- **On-line**
  - Samples are analyzed by taking out from the process stream in automated fashion, may or may not go back into the process stream

- **At-line**
  - Samples are analyzed by taking out from the process stream

**Traditional Analytics**
- Process Development
- Manufacturing
- Offline Testing
- Results

**Analytical Lab**
- Instruments are integrated
- Or Automated sampling
- Automated data analysis
- & Visualization

**PAT**
- Real time or near-real time analytical results
Strategy in Building Real Time Product Quality and Process Monitoring

“Monitoring As It Happens”

<table>
<thead>
<tr>
<th>Unit Operation</th>
<th>Attribute/Process Parameter</th>
<th>Choice of Analytical Technology</th>
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- Charge Variant Profile
- Glycan Profile
- Product Titer
- Nutrient and Metabolites

On-line & In-Line

- Product Concentration
- Impurity Clearance

At-line & In-Line
Strategy in Building Real Time Product Quality and Process Monitoring

“Real Time Acquisition vs. Monitoring”

Physical Sensors

Cyber-physical Systems

Real time Monitoring

Real time acquisition
Our PAT Vision

Unit Operation

Feedback/Feedforward Control

DCS

Sensors

Analyzers & Visualization

Repository

PAT Technology Landscape in BMS

- **(In)** Incyte Biomass
- **(On)** PATROL Titer
- **(On)** SegFlow-LC titer, amino acids, charge variants
- **(In)** Raman Titer, glucose
- **(In)** FTIR titer/concentration
- **(In)** FTIR concentration
- **(In)** FlowVPE concentration
- **(At)** ELLA HCP
- **(On)** PATROL HMW
- **(On)** PATROL

Case Studies to be presented

On-line Product Quality Monitoring by Multi - Attribute Chromatography

1. Size-exclusion chromatography
   Aggregation (HMW species)
2. Cation exchange chromatography
   Charge Heterogeneity
3. Amino Acid Analysis

Cell-free sample from Segflow

Protein A Column (purification/titer)

Strong Cation Exchange Chromatography
Agilent 2D-LC w/ High Resolution Peak-Cutting & Peak Parking: A Viable Option to Deal with Large 1st Dimension Peak Volume

Peak Cutting Capabilities for Agilent 2D-LC

Comprehensive Transfer

High Resolution Sampling

Conventional Heart Cutting

Heart Cutting & Peak Parking

Figure 1. Illustration of high-resolution sampling 2D LC.

Figure 3. Illustration of multiple heart-cutting 2D LC.
A representative IEX profiles of High Resolution peak cutting of 1\textsuperscript{st} dimension Protein-A & a 2\textsuperscript{nd} dimension IEX Chromatography.
An Alternate Approach to High Resolution Peak Cutting & Peak Parking to Enable Near Real-time Analysis

High Resolution Peak-cutting & Peak Parking

Advantages:
- Agilent 2D-LC provides the option to collect multiple fractions of 1st dimension Protein-A peak in multiple sample loops and inject one fraction at a time
- Agilent OpenLab CDS Chemstation software is capable to integrate the data from multiple fractions to integrated final results

Disadvantages:
- Long analysis time for analyzing multiple peak cuts, which is not in alignment with the rapid analysis required for of PAT

Alternative Approach with Flow Splitting

Advantages:
- Post flow-splitting of 1st dimension protein-A effluent can be achieved effortlessly with commercially available MS flow splitters
- Based on the peak volume of the 1st dimension protein-A chromatography, flow-splitters with different flow splitting ratios can be utilized
- With 1:10 flow-splitter, analysis time can be reduced by 10-fold

Disadvantages:
- No disadvantages
On-line Product Quality Analysis by 2D-LC CEX Multi - Attribute Chromatography

- Bioreactor titer obtained from 1D-LC acquisition
- A platform CEX method is applied at 2D-LC
- Multiple attributes, such as titer, charge profiles (acidic, main and basic groups) are measured near real-time by on-line 2D-LC technology
FT-IR & Chemometric Sensors at UFDF for Protein and Excipient Monitoring

Fourier-transform infrared spectroscopy (FT-IR)
- Vibrational Spectroscopy Technique
- Unique spectral bands for chemical bonds
- Measurement frequency - As fast as 10 sec.

Pre-processing
- Baseline correction
- Smoothing
- Derivatives

PLS Modeling
**Real Time Process Monitoring - Downstream**

**In-line FTIR**

Real Time Monitoring of Proteins & Excipients in UF/DF

**Automated,**
1. Data Piping
2. Data Analysis
3. Visualization

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**Data Piping**

**Data Analysis**

**Visualization**

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**Process monitoring software**

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**Modeling software**

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Holistic Process Understanding

CPPs
- Nutrients
- Metabolites
- Trace metals

CQAs
- Glycan Profile
- Charge Variants
- Titer

Process Control...

Quality by Design (QbD) Manufacturing

Descriptive
- What happened?

Diagnostic
- Why did it happen?

Predictive
- What will happen?

Prescriptive
- How to make it happen?
## Acknowledgement

### Analytical
- Dhanuka Wasalathanthri
- Letha Chemmalil
- Jay West
- Xia Xu
- Satish Sharma
- Neha Puri
- Sergey Voronov
- Chun Shao
- June Kuang
- Yan Gu
- Yutong Wu

### Upstream
- Michael Borys
- Matthew Rehmann
- Kyle McHugh
- Ziev Basson
- Emily Rittershaus
- Charles Hill

### Downstream
- Zhijun Tan
- Melissa Holstein
- Yuanli Song
- Hasin Feroz
- Jessica Hung
- Vivekh Ehampanaranathan
- James Angelo

### HTP & Scale Up
- Jongchan Lee
- Jeff Swanberg
- Derek Choy

### MS&T
- Amanda Lewis
- Eric Garr
- Eric Hodgman
- Juan Wang
- Juma Bridgewater
- Dong Yang
- Liming Yin
- Bryanne Zonghi
- Alexandra Tsoras

### Engineering Technologies
- Douglas Both
- Greg Lane
- Nobel Vale
- Georgios Pyrgiotakis
- Claudia Corredor

### IT
- Ed Keefe
- Steve Traylor
- Anthony Valbrun

### Process Engineering
- Mark Brancieri
- Amelia Bo

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