Automated Glucose Control in Ambr® Bioreactors Using Raman Spectroscopy

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Aims
- Demonstrate physical integration of and automated data integration for Raman spectroscopy with Ambr® bioreactors
- Generate data to build a Raman model for glucose concentration in a typical cell culture
- Build a Raman model for glucose concentration using SIMCA 16 software
- Demonstrate real-time feedback control of glucose in culture based on the Ambr® derived SIMCA 16 Raman model

1. BioPAT® Spectro in Ambr® Overview

2. BioPAT® Spectro Ambr® Key Features
- A user-replaceable Raman flow cell is integrated in the Ambr® Analysis Module, compatible with both Ambr® 15 Cell Culture and Ambr® 250 High Throughput
- Compatible with Raman Spectrometers and BioPAT® Spectro Raman probes from Kaiser Optical Systems and Torando-Spectra Systems
- Small sample volumes: 180 μL for Ambr® 15, 200 μL for Ambr® 250 High Throughput
- Fast cycle time ~30-15 min per sample
- Samples can be automatically spiked with a concentrated analyte stock solution prior to Raman analysis
- All key data from the Ambr® process, Raman and other integrated analyzers are automatically collated by the Ambr® Software
- Identical Raman probes and flow cell optical paths support direct model transfer to BioPAT® STR®
- Requires compatible Ambr® Analysis Module, Win32 Ambr® Control PC, separate SIMCA 16 license for creating models
- Suitable for typical mammalian fed-batch cell densities, microbial applications are not supported

3. Model Building Ambr® Run
- N = 16 Ambr® 250 High Throughput bioreactors
- Sartorius Cellca2 Process CHO producing mAb (includes glucose-containing feeds)
- Fully automated glucose feed to 5 g/L
- Daily integrated Nova FLEX2 glucose <5 g/L, automated glucose feed to 5 g/L
- JOO data points assayed by Flex2 then Raman, of which ~35 were spiked with 7.1 or 33.3 g/L glucose
- Data was automatically collated in the Ambr® Software and one file exported by the operator
- A separate copy of SIMCA 16 software was used for model building

4. Model Building Results
- A good Raman model was generated for glucose using only non-spiked samples (Fig. 4)
- Glucose model quality parameters are consistent with the best models in the literature
- Raman-based glucose control was similar to the FLEX2 assay
- Spiking was not required to generate a good glucose model but spiking can increase model range (Fig. 5)
- Spiking has been shown to improve range and model quality for other analytes e.g. lactate (data not shown)

5. Raman Based Ambr® Process Control
- Prior to the run, a glucose model previously built in SIMCA® was loaded into the Ambr® software
- Sartorius Cellca2 Process CHO producing mAb
- Integrated Raman and Nova FLEX2 analyzers
- A daily integrated Nova FLEX2 glucose <5 g/L, automated glucose feed to 5 g/L
- N = 4 bioreactors were automatically fed glucose based on integrated Raman predictions
- N = 4 bioreactors were automatically fed glucose based on integrated Nova FLEX2 analysis

6. Glucose Control Results
- Glucose was maintained in culture in the range of 1.8-4.8 g/L (glucose readings <5 g/L were due to glucose in feeds) (Fig. 6)
- Integrated Raman and Nova FLEX2 glucose assays were in very close agreement throughout (Fig. 6)
- Cell culture profiles (Fig. 7) were very similar for glucose control based on Raman or Nova FLEX2 assays
- BioPAT® Spectro in Ambr® enabled fully automated glucose control capabilities similar to other analytical techniques (Fig. 7)
- Further work will include confirmation of the Ambr glucose model performance in Sartorius’ Biostat STR® bioreactors (50-2000 L scale)

7. Conclusion
- Automated integration of Raman spectroscopy to Ambr® is now possible
- Fully automated data acquisition and alignment saves a significant amount of user time
- A high quality glucose model was generated covering a wide concentration range (up to 15 g/L)
- Ambr® systems enable robust model building due to a range of setpoints and spiking of samples
- Cell culture controlled by integrated Raman or Nova FLEX2 analyses showed very comparable glucose and VCD profiles

Figures:
- Figure 1: Schematic diagram of Ambr® Analysis Module with BioPAT® Spectro fitted to an Ambr® 250 High Throughput bioreactor system
- Figure 2: Ambr® Analysis Module with BioPAT® Spectro fitted to an Ambr® 15 bioreactor. Inset box: User-replaceable BioPAT® Spectro flow cell
- Figure 3: Workflow diagram for BioPAT® Spectro in Ambr®
- Figure 4: Glucose control results for non-spiked samples
- Figure 5: Glucose model, including spiked samples (N ~ 280 total)
- Figure 6: Glucose model excluding spikes samples (N ~ 250 total)
- Figure 7: Glucose concentration in a fed-batch culture measured by both integrated FLEX2 and Raman prediction. Not bioreactor shown for clarity.