Simplifying Progress

Facilitated Process Scalability Using a Novel Raman Spectroscopy Integration

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Aims

- Demonstrate hardware comparability of the BioPAT® Spectro interfaces in Ambr® and Flexsafe STR® and existing immersion probes
- Demonstrate model scalability of a cell culture process from Ambr® to the production size Flexsafe STR® bags

Key Features of BioPAT® Spectro in Ambr® and Flexsafe STR®

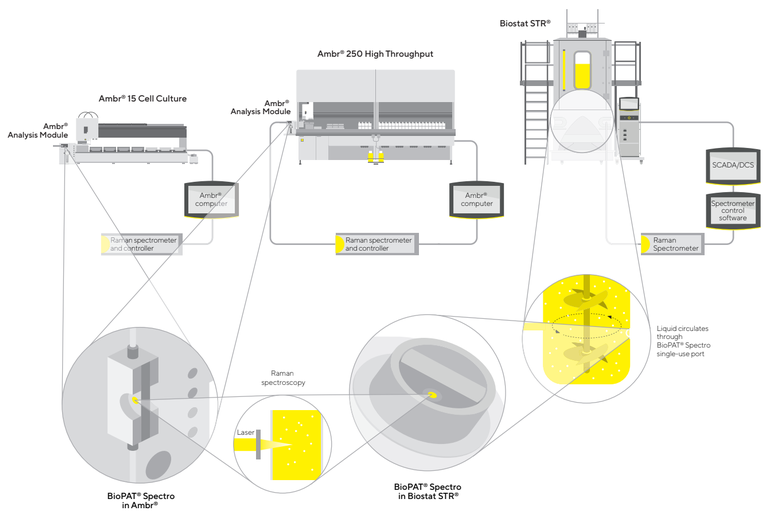
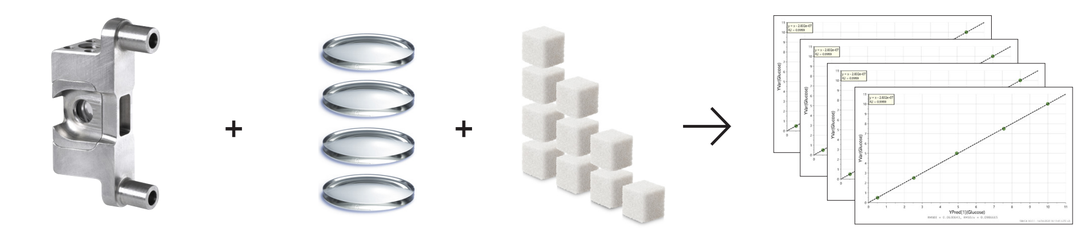


Figure 1: *Schematic Diagram of the BioPAT® Spectro Raman Spectroscopy Integration in Ambr® 15 Cell Culture, Ambr® 250 High Throughput And Biostat STR® Single-Use Bioreactor Systems.*

Hardware Scalability Tested in a Lab Set-Up

Model Building Using the Ambr® Flow Cell

- Ambr® flow cell with four different optical windows
- Raman spectra were acquired for each combination with glucose concentrations between 0.5 and 10 g/L
- Four corresponding glucose models were built using SIMCA®



BioPAT® Spectro flow cell from Ambr® | 4 Windows | Glucose 0.5-10 g/L | 4 Ambr® Glucose Models

Figure 3: *Schematic of the Experimental Setup: 4 Different Windows Were Tested in the BioPAT® Spectro Ambr® Flow Cell and Glucose Models Were Built.*

Model Transfer From the Ambr® Flow Cell to the Flexsafe STR® Port and an Immersion Probe

- Single-use ports of the Flexsafe STR® with the same four optical windows from above
- Raman spectra were acquired for each combination with glucose concentrations between 0.5 and 10 g/L
- A conventional immersion probe used to acquire spectra in a beaker with 0.5 – 10g/L glucose
- Glucose multi-variate models generated with the Ambr® flow cells were used to predict the glucose concentrations

4 Ambr® Glucose Models | 2 Flexsafe STR® Spectro Ports | 4 Windows | Glucose 0.5 – 10 g/L

Figure 4: *Schematic of the Experimental Setup: The 4 Different Ambr® Glucose Models From Above Were Applied to Predict the Glucose Concentration Of 4 Different SU Port/Window Combinations.*

Model Transfer Results

- Ambr® models led to a consistent and precise prediction of the glucose concentrations in the Flexsafe STR® ports and the immersion probe
- The average prediction error across all predictions was 0.23 g/L and is comparable to the literature.

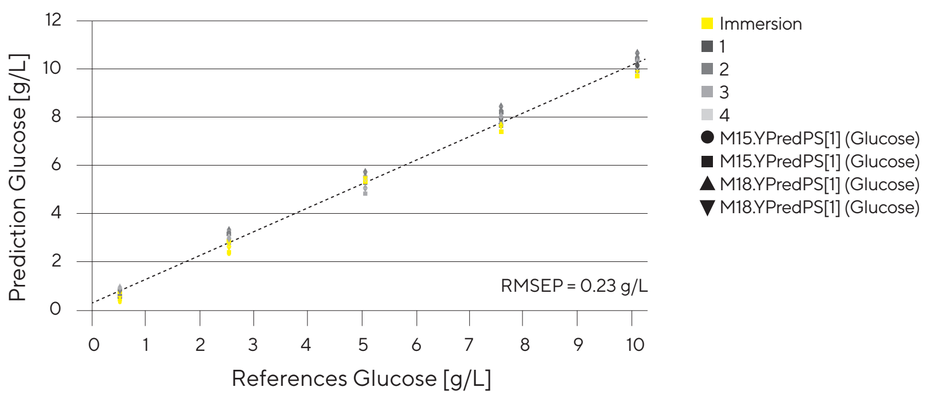


Figure 5: *Graph Showing the Prediction of the 4 Ambr® Models Of the Glucose Concentrations Measured With The STR SU Ports and a Conventional Immersion Probe.*

BioPAT® Spectro Key Features

- Scalable Raman Spectroscopy platform from 15 mL to 2000 L
- Ambr® Analysis Module with replaceable Raman flow cell
- Compatibility: Ambr® 15 Cell Culture, Ambr® 250 High Throughput
- Fully automated data acquisition, alignment and model application in Ambr®, with automated spiking
- Fully integrated and sterilized single-use port in Flexsafe STR® 50 – 2000 L
- Port qualified for GMP manufacturing, shields measurement from ambient light and decouples the measurement from process conditions such as gassing and stirring
- Raman spectroscopy: Compatibility with Raman spectrometers from Endress+Hauser Optical Systems and Tornado Spectral Systems
- Facilitated model transfer from Ambr® to Biostat STR® and vice versa due to identical optical design and the use of the same fiber optic probe

Figure 2: *Left: Ambr® Analysis Module With BioPAT® Spectro. Inset Box: User Replaceable BioPAT® Spectro Flow Cell. Right: Flexsafe STR® Bag Installed in the Biostat STR® With the BioPAT® Spectro Single-Use Port. Inset Box: The Single-Use Port.*

Process Scalability Tested in a Cell Culture Process

Model Building Using the Ambr® 250 High Throughput

- N= 16 Ambr® 250 High Throughput bioreactors
- Sartorius' Cellca 2 Process CHO producing mAb
- Integrated Raman and Nova Flex 2 analyzers
- 200 data points assayed by Flex 2 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 manually exported to SIMCA®
- SIMCA® 16 software was used for multivariate model building

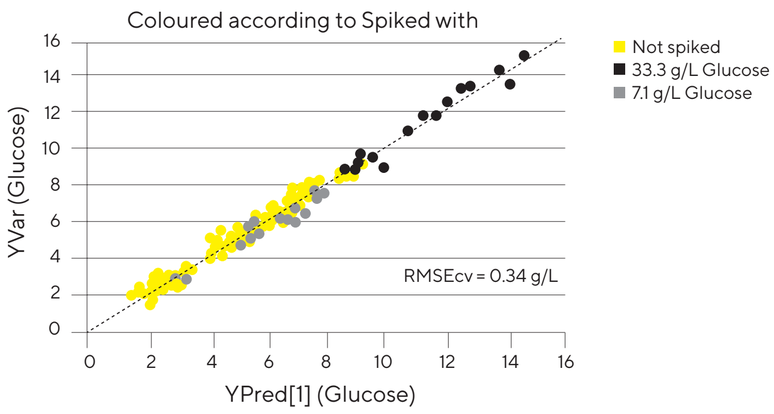


Figure 6: *Graph Showing the Performance of the Glucose Model Built in Ambr® 250 When Applied to Ambr® 250 Vessels That Did Not Contribute to The Model Building Data. Automated Spiking Was Performed With Two Different Glucose Stock Solutions.*

Model Transfer From Ambr® 250 to 200 L Biostat STR®

- Cellca 2 process was run in a 200 L Flexsafe STR® bag, at a different Sartorius site using a spectrometer and fiber optic probe with different serial numbers
- BioPAT® Spectro SU port was used, which comes integrated and sterilized inside the bag
- The Ambr® mutli-variate model was applied to the spectra without further modifications to predict glucose

Model Transfer Results

- The Ambr® model on the Biostat STR® data predicted the glucose profile and identifies feeding events
- Predictions agreed with offline measured glucose concentration
- Improved results are to be expected when updating the Ambr® Raman model with spectra acquired in at least one Biostat STR® run, which we recommend

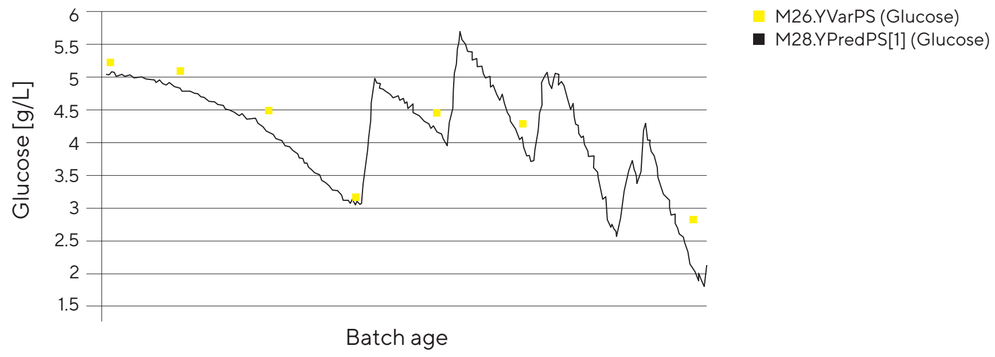


Figure 7: *Graph Showing the Prediction of the Raman Glucose Model Built in Ambr® of the Glucose Concentration in the Biostat STR® 200.*

Conclusions

- Raman models transfer between the hardware used to acquire Raman spectra in the Ambr® and Biostat STR® systems in a laboratory set-up
- The BioPAT® Spectro optical interfaces are comparable to conventional immersion probes and models can be transferred between them in a laboratory set-up
- The Raman glucose model for the Cellca2 process in the Ambr® 250 High Throughput acquired by the BioPAT® Spectro integration is readily transferable to the same process in the Flexsafe STR® bag using the BioPAT® Spectro SU port
- This indicates that models built for other processes and analytes are also likely to scale well in the BioPAT® Spectro platform