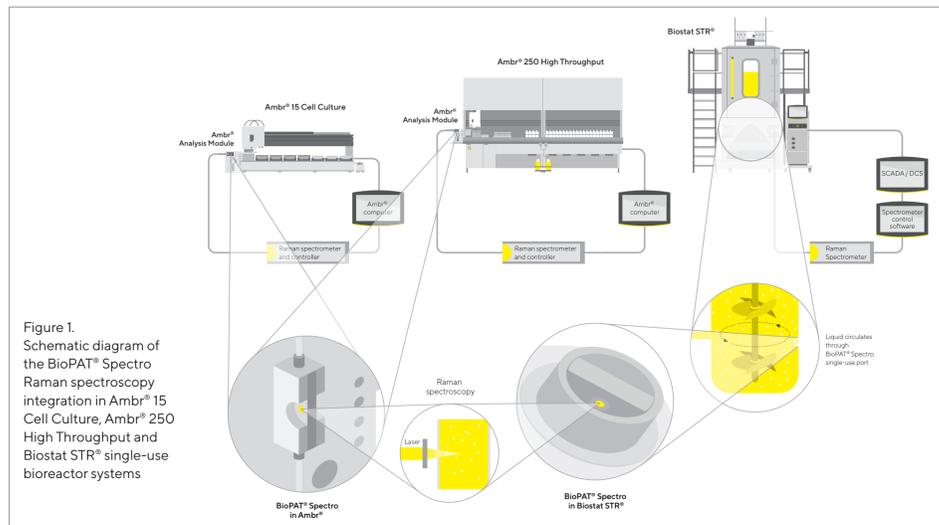# Facilitated Process Scalability Using a Novel Raman Spectroscopy Integration

Michael Sibley[1], Angus Woodhams[1], Marek Hoehse[2], Svea Cheeseman[1]*

1 Sartorius Royston, UK. 2 Sartorius Göttingen, Germany. *svea.cheeseman@sartorius.com

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
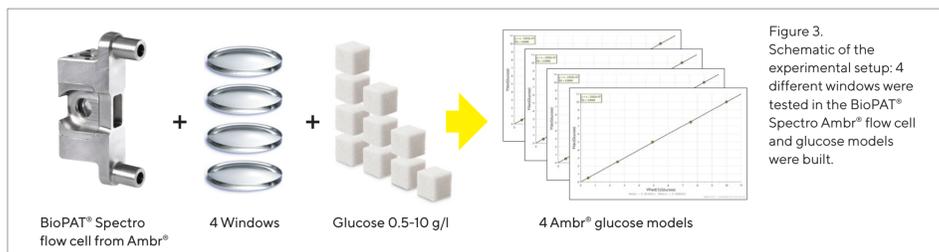
BioPAT® Spectro Key Features

- Scalable Raman Spectroscopy platform from 15 mL to 2000L
- 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 Kaiser 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.

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
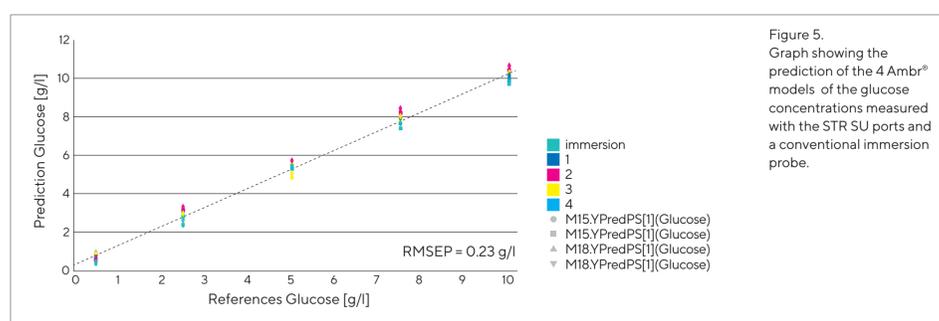- Four corresponding glucose models were built using SIMCA®

Figure 3.
Schematic of the experimental setup: 4 different windows were tested in the BioPAT® Spectro Ambr® flow cell and glucose models were built.

BioPAT® Spectro flow cell from Ambr®   +   4 Windows   +   Glucose 0.5-10 g/l   →   4 Ambr® glucose models

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

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.

4 Ambr® glucose models   +   2 Flexsafe STR® Spectro Ports   +   4 Windows   +   Glucose 0.5-10 g/l
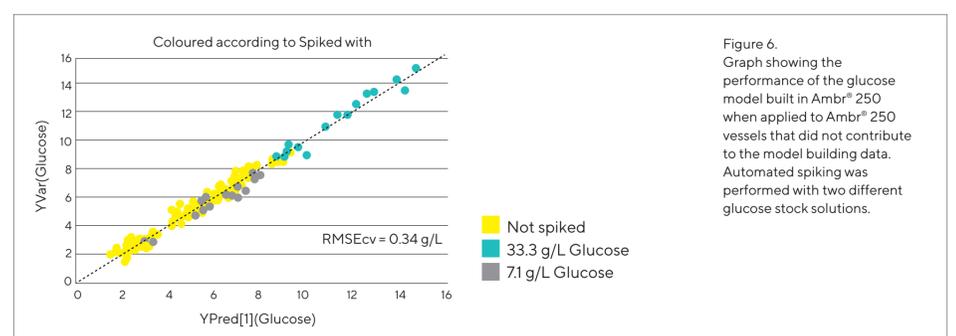
Model transfer results
- Ambr® models led to a consistent and precise prediction of the glucose concentrations in the Flexsafe STR® ports and the immersion probe
- The average prediction error across all predictions was 0.23 g/L and is comparable to the literature.

RMSEP = 0.23 g/l

immersion
1
2
3
4
M15.YPredPS[1](Glucose)
M15.YPredPS[1](Glucose)
M18.YPredPS[1](Glucose)
M18.YPredPS[1](Glucose)

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.

Process Scalability Tested in a Cell Culture Process

Model building using the Ambr® 250 High Throughput
- N= 16 Ambr® 250 High Throughput bioreactors
- Sartorius' Cellca2 Process CHO producing mAb
- Integrated Raman and Nova FLEX2 analyzers
- 200 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 manually exported to SIMCA®
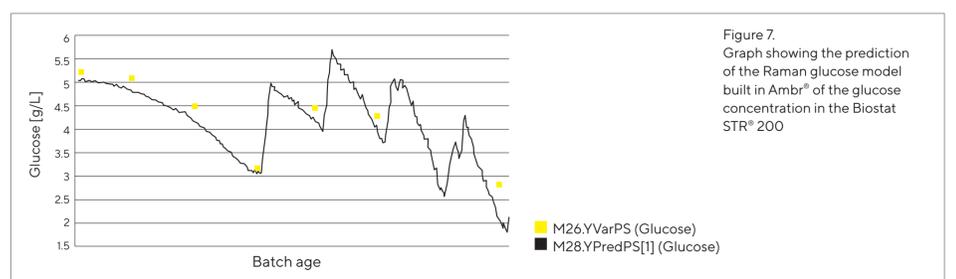- SIMCA 16 software was used for multivariate model building

Coloured according to Spiked with

RMSEcv = 0.34 g/L

Not spiked
33.3 g/L Glucose
7.1 g/L Glucose

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 200L Biostat STR®
- Cellca 2 process was run in a 200L 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

Batch age

M26.YVarPS (Glucose)
M28.YPredPS[1] (Glucose)

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