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Glucose and Lactate Monitoring with PTRam

Introduction

Raman spectroscopy is a valuable technique for process analytical technology (PAT) in the pharmaceutical industry due to its capacity for non-destructive, real-time measurements, as well as its ability to be implemented for online and inline monitoring. Prior to large-scale implementation of a new process or product, studies are performed in labs and at small-scale pilot plants to assess feasibility.

Biopharmaceuticals manufacturing is an industry that has adopted Raman spectroscopy measurements for PAT studies, particularly for cell culture and fermentation monitoring. Raman spectroscopy can be used to monitor the growth media components (including glucose) and metabolic products (such as lactate) in cell culture upon development of a multivariate statistical model. Figure 1 shows the Raman spectra of pure glucose and pure lactate, which have sharp and distinct bands.

In this application note, we'll demonstrate the feasibility of monitoring glucose and lactate in a dynamic system using B&W Tek's PTRam process development Raman analyzer.

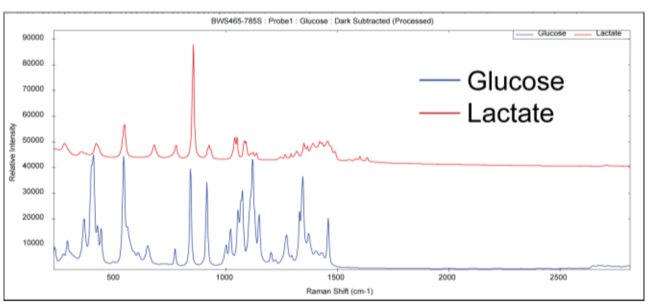

Figure 1. Raman spectra of glucose and lactate powders (spectra are manually offset for clarity)

Static Experiment

Twenty-five aqueous solutions containing variable amounts of glucose and lactate were gravimetrically created. Glucose concentrations ranged from 0 - 4.5 g/L, and lactate concentrations ranged from 0 - 4.4 g/L.

A fiber optic probe with an immersible fused silica shaft (RIS100-FS) was used to measure all solutions. The laser power used was 100% of the maximum laser power (≈340 mW), and all spectra were collected with an 18 s integration time. Table 1 shows the technical specifications of the PTRam. Metrohm Vision software was used to acquire all calibration spectra.

Table 1. PTRam specifications

Model	BWS476-785H
Laser source	785 nm. Full power 340 mW at sample. Power level adjustable by software
Spectral range	150-2800 cm^{-1}
Sample channels	1
Weight	32 lb (14.5 kg)
Operating temperature	15 ˚C to 30 ˚C
Raman shift calibration	Self-calibrating with internal reference
Operating software	Vision©

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Calibration model

Vision software was used to create calibration models for prediction of glucose and lactate. Figure 2a shows the calibration data acquired by the PTRam. All spectra are relative intensity-corrected against a NIST 2241 standard. Figure 2b shows the preprocessed calibration spectra for the glucose model. The preprocessing applied is a Savitzky-Golay first derivative and a standard normal variate (SNV) using a range of 1066-1811 cm^{-1}. A model for lactate was created using a Savitzky-Golay second derivative with a SNV using a range of 635-940 cm^{-1} and 1066-1811 cm^{-1}.

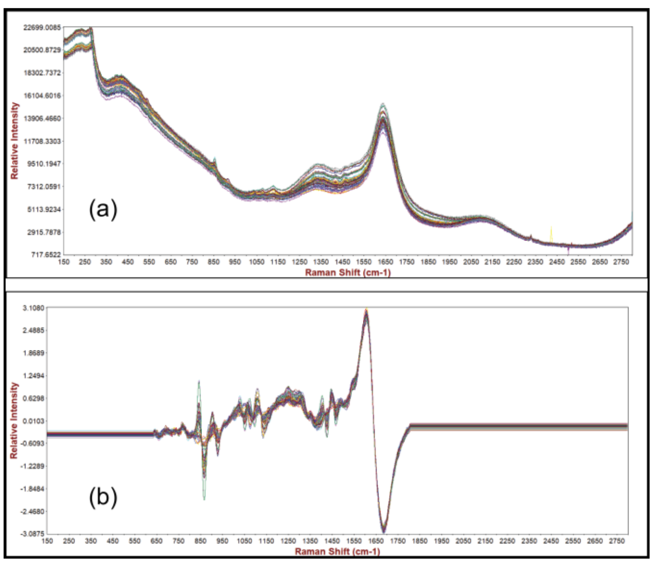

Figure 2. (a) Raw data of glucose and lactate solutions and (b) calibration spectra with preprocessing algorithms applied to the glucose model

Partial least square (PLS) models were created in Vision to model the glucose and lactate constituents with cross-validaiton with four segments. Figure 3 shows the calculated vs. lab data plots for glucose and lactate. Each model uses three factors. Parameters for model linearity and accuracy are also shown for both constituents.

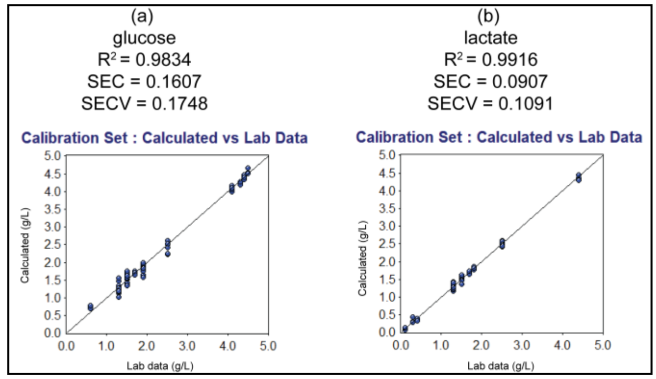

Figure 3. Calculated vs. lab data plots for (a) glucose and (b) lactate. Parameters for linearity and accuracy are also shown.

Dynamic Process Experiment

To demonstrate the performance of PTRam for monitoring the culture solution of a cell growth application, a starting aqueous solution of 5 g/L glucose was gravimetrically prepared. A fiber optic probe with an immersible fused silica shaft (RIS100-FS) was used to monitor the solution.

To simulate the consumption of glucose by cells and the production of metabolic lactate, multiple volumes of aqueous lactate solutions were added to the starting solution at 10 minute intervals. To simulate the replenishing of glucose and leveling off of lactate, volumes of the monitored solution were removed and replenished with amounts of an aqueous solution containing a high concentration of glucose and low concentration of lactate.

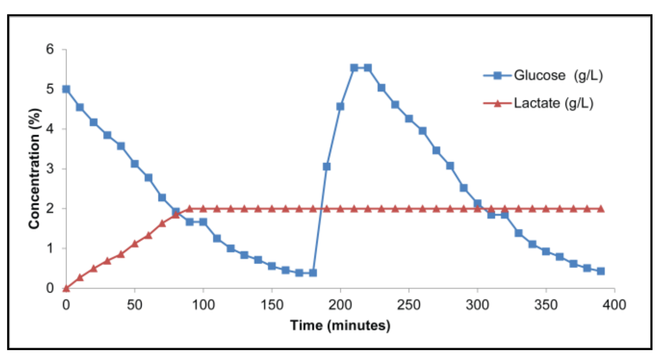

Figure 4. Theoretical curves for dynamic glucose and lactate measurements generated in Microsoft Excel

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Finally, various amounts of aqueous lactate were again added to the solution to simulate further consumption of the glucose.
The concentrations of glucose and lactate were monitored over 6.5 hours. Figure 4 shows the theoretical curves for lactate and glucose concentrations with time.
A routine analysis operation method was created in the Vision software for real-time prediction of the glucose and lactate levels based on the calibration models. Spectra were collected with an integration time of 18 s and a laser power of 100% of the maximum integration time (≈ 340 mW).
Figure 5 shows the combined trend chart for glucose and lactate concentrations generated during the dynamic experiment, which matches the theoretical curve quite closely. Table 2 shows the statistics from the routine analysis prediction, including the standard error of prediction (SEP) and bias.

Conclusion

The PTRam is a high-performance portable Raman system designed for monitoring processes like cell culture and fermentation in analytical labs and pilot plant settings. Together with Metrohm's Vision software, the PTRam can be used to acquire real-time results in industries such as pharmaceuticals, chemicals, and polymer manufacturing!

For more information:

Go to https://bwtek.com/products/ptram/.

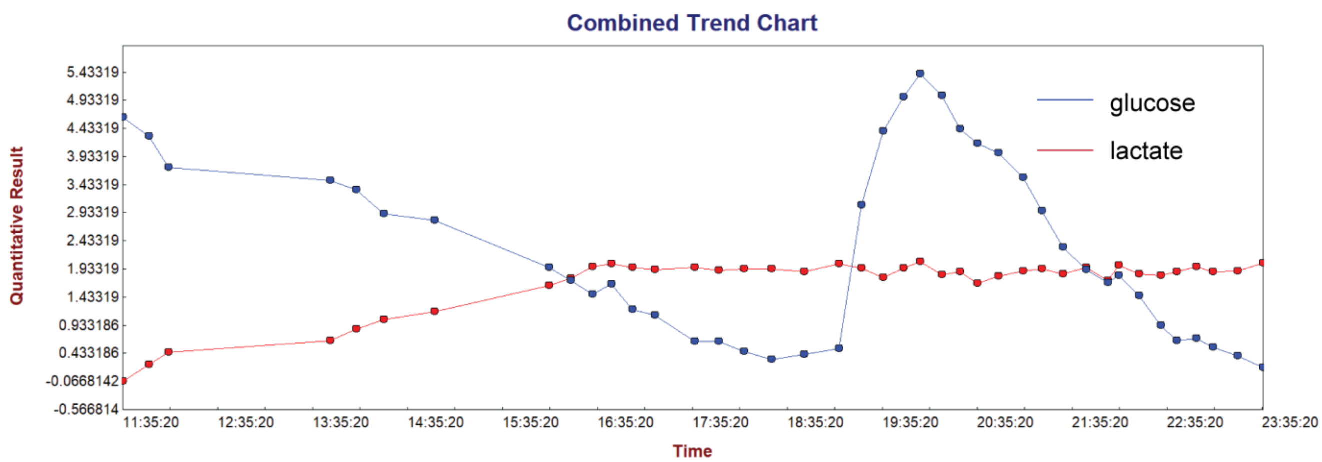

Figure 5. Combined trend chart for dynamic glucose and lactate measurements generated automatically in real-time from the Vision software.

Table 2. Prediction accuracy parameters			
Glucose		Lactate	
Minimum concentration	0.38 g/L	**Minimum concentration**	0.0 g/L
Maximum concentration	5.5 g/L	**Maximum concentration**	2.0 g/L
Bias	-0.1349	**Bias**	-0.0849
SEP	0.2009	**SEP**	0.1166

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