Rapid Development of a 9 component Multivariate Calibration for the analysis of Metabolites in Chemically Defined Cell Culture Media

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This study assessed the performance of the HyperFlux™ PRO Plus Raman Spectroscopy System (Tornado Spectral Systems) for the quantitative analysis of biochemical components in a simplified chemically defined pseudo growth medium for mammalian cell culture. An experiment was designed to evaluate the ability of Raman Spectroscopy to directly measure individual components in a complex mixture at concentrations at or below the limit of quantification of conventional Raman spectrometers. A set of samples with varying amounts of glucose, lactate, glutamine, glutamate, ammonium, arginine, histidine, leucine, and phenylalanine were prepared so that covariance between components was close to zero. The spectral collection and model development were completed in one day. The sample spectra were collected in a morning using fast acquisition times and promising calibrations were developed in the afternoon using basic pretreatments such as derivatives and normalisation.

Background

Real-time analysis of biochemical biomarkers in bioreactors using Raman Spectroscopy has been widely described in the literature.[1,2,3,4,5,6] Having the ability to accurately monitor the consumption of starting materials, the formation of waste products, as well as estimate the rates of cell growth, apoptosis (viable cell count and total cell count) and protein expression in real time would provide valuable opportunities to better control the process and thereby improve product yield and consistency.

Raman spectroscopy has not yet been widely implemented in industrial bioreactors and this is due mainly to the complexity of the sample under analysis. There are many interfering signals arising from a cocktail of metabolites which makes calibration model development challenging.[7] A second factor is that in bioprocesses, there are high levels of collinearity between variables to be predicted, making it unclear sometimes whether the compounds of interest are being measured directly or via some inferential means which in turn has implications for scalability. Finally, the sensitivity of Raman instruments can be an issue as sample concentrations tend to be very dilute and Raman spectrometers are operating at the lower end of their capabilities. Measurement times of 10-13 minutes to obtain a signal are quoted in the literature[1,5].

In this analysis, a design space was generated to produce a multivariate calibration set for a chemically defined growth medium which contained no correlations between variables. The samples were then synthesised in the laboratory and analysed using the HyperFlux™ PRO Plus 785 nm Raman Spectroscopy System.

Method

Sample Preparation:

Using an experimental design which ensured orthogonality between the variables,[8,9] a design space was mapped out with nine variables and five levels. A sample covariance plot between two variables is shown in Figure 1. Similar graphs could be plotted for all combinations of variables.
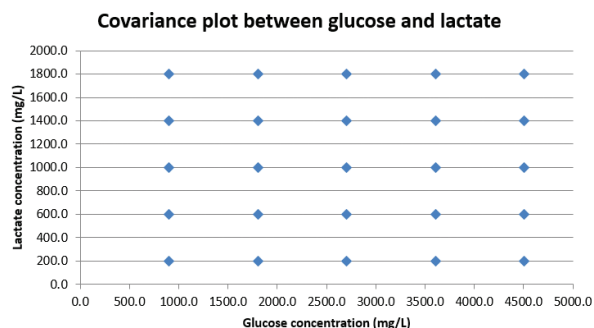

FIGURE 1: An Example Covariance plot for Lactate vs. Glucose

The variable components in the samples were glucose, lactate, glutamine, glutamate, ammonium, arginine, histidine, leucine, and phenylalanine. The invariant components in the samples were buffer and serum and these were present at a high concentration with pH maintained at 7.0.

Individual stock solutions were prepared for the nine varying components by dissolving a weighed amount of chemical in a buffer solution at pH 7.0. Two sets of samples were prepared by mixing the appropriate volume of stock solutions together. The samples from the second set were complemented with 10% serum.

The final concentration of the samples ranged from 0.9 to 4.5 g/L (900-4,500 ppm) for glucose, from 0.2 to 1.8 g/L (200-1,800 ppm) for lactate and from 0.1 to 1 g/L (100-1,000 ppm) for glutamine, glutamate, ammonium, arginine, histidine, leucine, and phenylalanine.

Spectral acquisition:

Triplicate spectra were collected on the HyperFlux™ PRO Plus Raman Spectroscopy System using the acquisition parameters detailed below:

Laser Intensity (at source):   495 mW

Exposure Time: 3 seconds

Accumulations: 15

Total acquisition time: 45 seconds

Raw spectra between 1800 and 800 cm^{-1} are shown in Figure 2 and it can be seen that the signal to noise ratio is high despite the short acquisition time of 45 seconds.

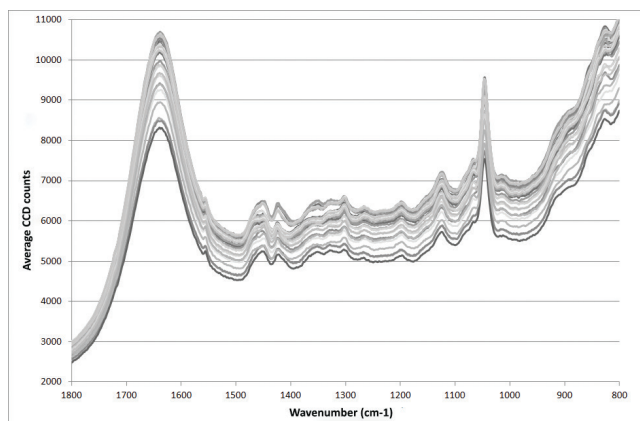*FIGURE 2: Raw spectra collected using Tornado's HyperFlux™ PRO Plus Raman Spectroscopy System*

Calibration model development:

Individual PLS calibration models were developed for each of the nine components using Bruker OPUS Software.

Results and Discussion:

The performance of the calibrations is summarised in Table 1, and the test set data are plotted in Figure 3.

TABLE 1: R2 and RMSEP of the calibration models

		R^2	RMSEP [g/L (ppm)]
A	Glucose	99.87	0.0530 (53.0)
B	Lactate	99.35	0.0553 (55.3)
C	Glutamine	96.18	0.0829 (82.9)
D	Glutamate	96.47	0.0708 (70.8)
E	Histidine	97.89	0.0389 (38.9)
F	Leucine	99.38	0.0265 (26.5)
G	Arginine	89.87	0.1030 (103)
H	Phenylalanine	99.81	0.0142 (14.2)
I	Ammonium	99.87	0.0121 (12.1)

Phenylalanine and ammonium salts were the best performing calibrations while the calibration for arginine had the highest error.

It will be possible to further improve the precision of the measurements in general by increasing the number of measurements taken. For this analysis the measurement time was 45 seconds. For use in bioreactor a sampling frequency of 15 minutes would be considered sufficient so there is a lot of scope to increase acquisition time. Critically for dynamic processes, an acquisition time of 3 seconds should be achievable.
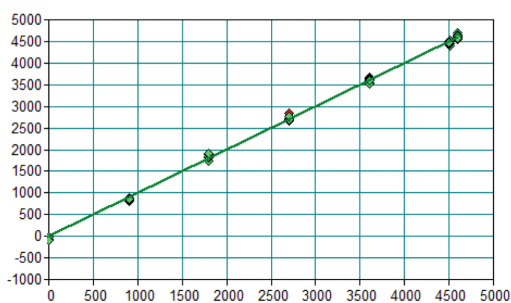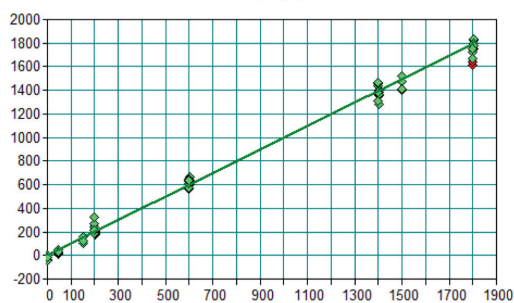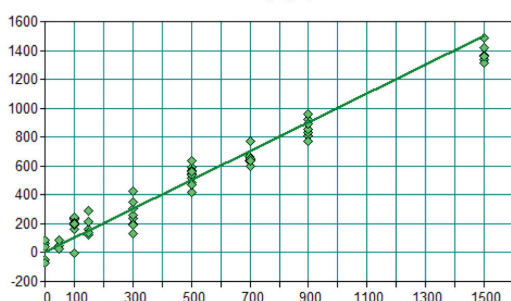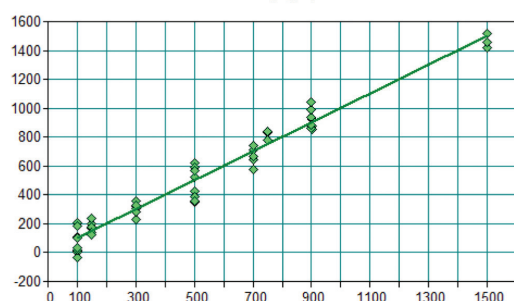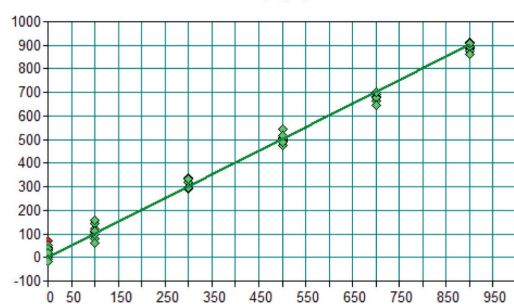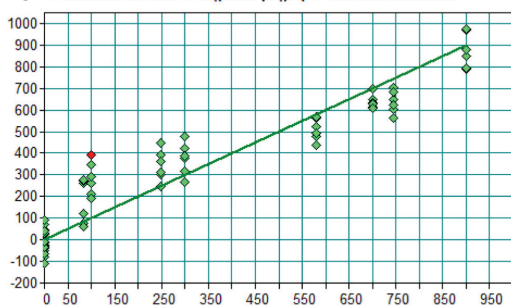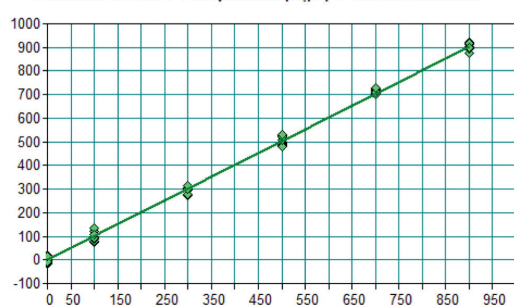
A Prediction vs True / Glucose [mg/L] / Test Set Validation

B Prediction vs True / Lactate [mg/L] / Test Set Validation

C Prediction vs True / Glutamine [mg/L] / Test Set Validation

D Prediction vs True / Glutamate [mg/L] / Test Set Validation

E Prediction vs True / Histidine [mg/L] / Test Set Validation
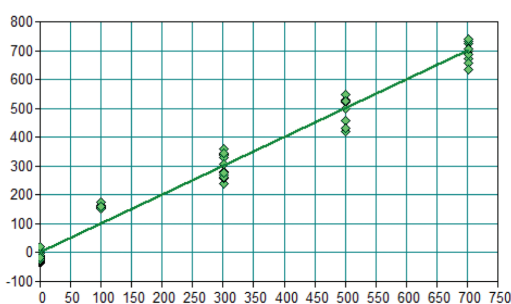
F Prediction vs True / Leucine [mg/L] / Test Set Validation

G Prediction vs True / Arginine [mg/L] / Test Set Validation

H Prediction vs True / Phenylalanine [mg/L] / Test Set Validation

I Prediction vs True / Ammonium [mg/L] / Test Set Validation
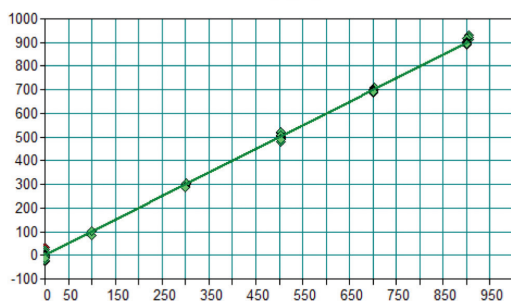
FIGURE 3: Predicted vs. True diagram of the test set validation models for Glucose (A), Lactate (B), Glutamine (C), Glutamate (D), Histidine (E), Leucine (F), Arginine (G), Phenylalanine (H) and Ammonium (I).

Conclusion:

Raman Spectroscopy has the ability to be used at a multicomponent sensor to monitor substrates and products of metabolism bioreactors. In this study, we used an experimental design that ensured orthogonality between variables. We believe we have demonstrated that with the HyperFlux™ PRO Plus Raman Spectroscopy System, it is possible to directly and reliably analyse many critical components down to at least 0.1 g/L (100 ppm) concentration in complex mixtures.

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About Sanofi:

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Tornado Spectral Systems designs, manufactures, and sells dispersive optical spectrometers primarily for Raman spectroscopy and spectral-domain optical coherence tomography. Tornado's HyperFlux spectrometers deliver significantly enhanced sensitivity by using a patented high-throughput virtual slit (HTVS™) to eliminate the physical slit of a conventional spectrometer and avoid signal losses while maintaining high spectral resolution.

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