On-line Recalibration of FTIR Measurements Using Metabolite Spiking and Dynamic Orthogonal Projection

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Introduction

Fourier-transform infrared (FTIR) spectroscopy
- increasingly used in bioprocess monitoring and control applications
- characteristic absorbance peaks in the *fingerprint* region (1800-800 cm^{-1})
- non-invasiveness, *in-situ* sterilizability
- ability to provide real-time concentration profiles for several metabolites simultaneously

Stochastic drift of the measured spectra in time
- The FTIR signal is sensitive to short-term fluctuations and long-term drift
- Using dual-beam instruments, the drift effect can be eliminated by taking a background spectrum before each measurement
- Using single-beam instruments, mathematical model correction methods can be applied

Methodology for single-beam instruments

To maintain the online robustness of the calibration model and to reduce the effect of signal drift,
- planned pulse additions of known amounts of monitored analytes are injected into the culture medium
- a novel model adaptation method known as Dynamic Orthogonal Projection (DOP) [1] is used to fine-tune the calibration model on-line

Experimental Setup and Calibration

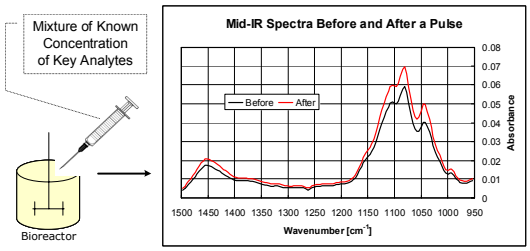
Application: anaerobic batch culture of *Saccharomyces cerevisiae* CBS 426 on glucose in a 2L laboratory RC1 calorimeter

Monitoring: single-beam FTIR (ReactIR 4000 from Mettler-Toledo) equipped with a flow-through cell. Single water background spectrum collected at beginning of experiment

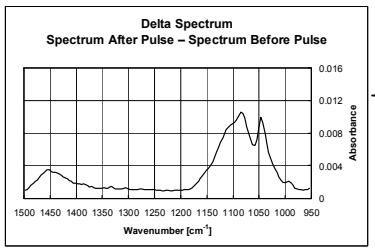
- A calibration model for five analytes was developed using mean-centered data, Partial Least Squares (PLS) and a 7-level multivariate design [2]
- The choice of the number of PLS factors was based on Predictive Residual Error Sum of Squares (PRESS) plots of leave-one-out cross-validation

Analyte	Concentration Range [g/L]	Spectral Range [cm^{-1}]	PLS Factors	Standard Error of Calibration (SEC) [g/L]
Glucose	0 - 25	1500 - 950	12	0.347
Ethanol	0 - 25	1300 - 950	8	0.327
Ammonium	0 - 2	1500 - 950	4	0.037
Phosphate	0 - 4	1500 - 950	13	0.042
Glycerol	0 - 4	1150 - 950	7	0.222

A novel on-line model adaptation approach



Delta spectrum
- corresponds to the pulse-injected mixture
- serves as an on-line reference measurement



DOP Algorithm [1] using Metabolite Spiking

1. Scale each pulse composition to fall into the calibration design (linearity between analyte concentration and absorbance to be checked)

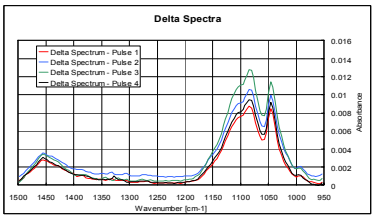
2. Estimate the delta spectrum that should have been obtained in the absence of signal drift from the calibration set using a Gaussian kernel function of the scaled pulse composition

3. Compute the orthogonal correction from the difference between the augmenting set of estimated and on-line delta spectra (additive drift assumed)

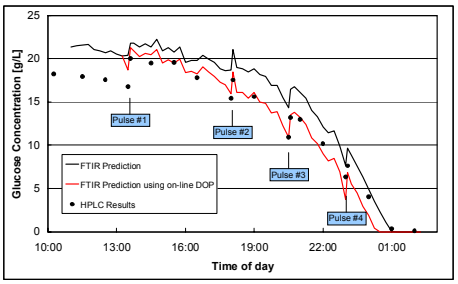
4. Recalibrate the model based on the orthogonally corrected calibration data sets

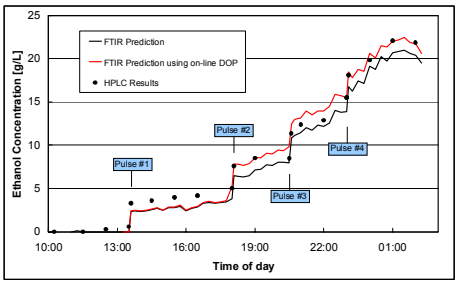
Experimental Results

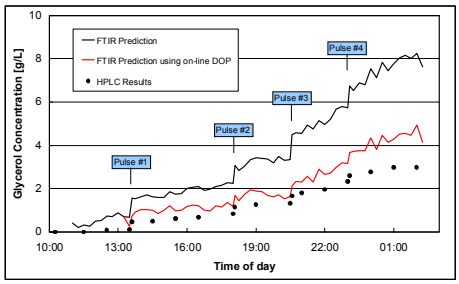
Four mixture pulses were injected with a composition corresponding to a multiple (1/5) of the calibration design centre:
- 2.5 g/L of Glucose,
- 2.5 g/L of Ethanol,
- 0.2 g/L of Ammonium,
- 0.4 g/L of Phosphate,
- 0.4 g/L of Glycerol



- Signal drift was observed in the resulting delta spectra (see figure to the left)
- Linearity between mixed analyte concentration and absorbance was verified by Principal Component Analysis (PCA) of 8 mixtures with linearly dependent compositions (1 significant factor observed)
- Each pulse composition and delta spectrum were multiplied by a factor of 5 to scale it to the mean of the calibration data set
- Following each pulse, the calibration model was corrected by DOP in real-time to reduce the effect of signal drift
- Improved prediction performance was achieved for glucose, ethanol and glycerol (see figures and table below)







- The application of DOP lead to a significant reduction in the standard error of prediction (SEP) for each analyte
- The results were compared to the commonly used In-Process Standard (IPS) method [3], where the calibration matrix is augmented by on-line reference standards

Analyte	SEP without DOP [g/L]	SEP with DOP [g/L]	Improvement with DOP	Improvement with IPS
Glucose	2.600	1.662	**36.1%**	17.1%
Ethanol	1.149	0.833	**27.5%**	9.1%
Ammonium	0.187	0.144	**23.1%**	1.3%
Phosphate	0.646	0.456	**29.4%**	35.9%
Glycerol	2.717	0.783	**71.2%**	33.4%

Conclusions and Future Work

- On-line reduction of SEP has been achieved by spiking the culture with known amounts of analytes and using DOP to correct the calibration model.
- The main advantage of this novel approach is that the reference samples did not need to be analyzed by off-line methods; thus, the model adaptation is performed in real-time.
- Future work will include applying the DOP approach to a fed-batch culture where continuous feeding is replaced by substrate pulses.

[1] Zeaiter, M., J. M. Roger, et al. (2005). "Dynamic Orthogonal Projection. A new method to maintain the on-line robustness of multivariate calibrations. Application to NIR based monitoring of wine fermentations." Preprint submitted to Elsevier Preprint.
[2] Brereton, R. (1997). "Multilevel multifactor designs for multivariate calibration." The Analyst, 122, p. 1521–1529.
[3] Kornmann, H. (2003). Monitoring and control of exopolysaccharides production by *Gluconacetobacter Xylinus* I-2281 using *in-situ* mid-infrared spectroscopy. PhD thesis, Swiss Federal Institute of Technology, Lausanne.

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