Monitoring of fermentation broths operated with *E. coli* cells— what is measured by near infrared spectroscopy?

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Introduction

The application of modern process analytical technologies (PAT) is increasing in pharmaceutical production and quality control, which drives the industry to science-based standards for manufacturing controls. The use of PAT/QbD (quality by design) tools and principles offers the pharmaceutical industry the possibility to increase product quality consistency, and to reduce product risk through increased knowledge/understanding and optimised process control.[1,2] PAT is especially important in the biopharmaceutical process development and manufacturing, where real-time measurements, application of chemometric tools, data modelling, process modelling, product and process design and optimisation are essential.[3–8]

The aim of our work was to execute a feasibility study on whether near infrared (NIR) technology is applicable for monitoring a fermentation process executed with *E. coli* cells producing a particular protein-based active pharmaceutical ingredient (API) molecule. Fermentation broths were monitored by fibre-optic bundles where the amount of biomass, substrates, nutrients and the status of fermentation process were detected.

Experimental

E. coli cells were cultivated in BIOSTAT B plus Twin (Sartorius Stedim Biotech, Aubagne, France) bioreactors with 1 litre working volume. Reference samples were withdrawn every hour to provide wet chemistry values. Acetate (Ace), glycerol (Gly) and ammonium (NH4+) measured by BioProfile 300 (Nova Biomedical, Waltham, MA, USA). Optical density (OD) measured by Ultrospec 500 pro (Amersham Biosciences, Uppsala, Sweden) visible spectrophotometer at λ = 600 nm.

NIR spectra were collected at every 5th minute throughout the 21 hour-long cultivation by an XDS Process Analytic MicroBundle Multiplexer (Foss NIRSystems, Silver Spring, MD, USA)

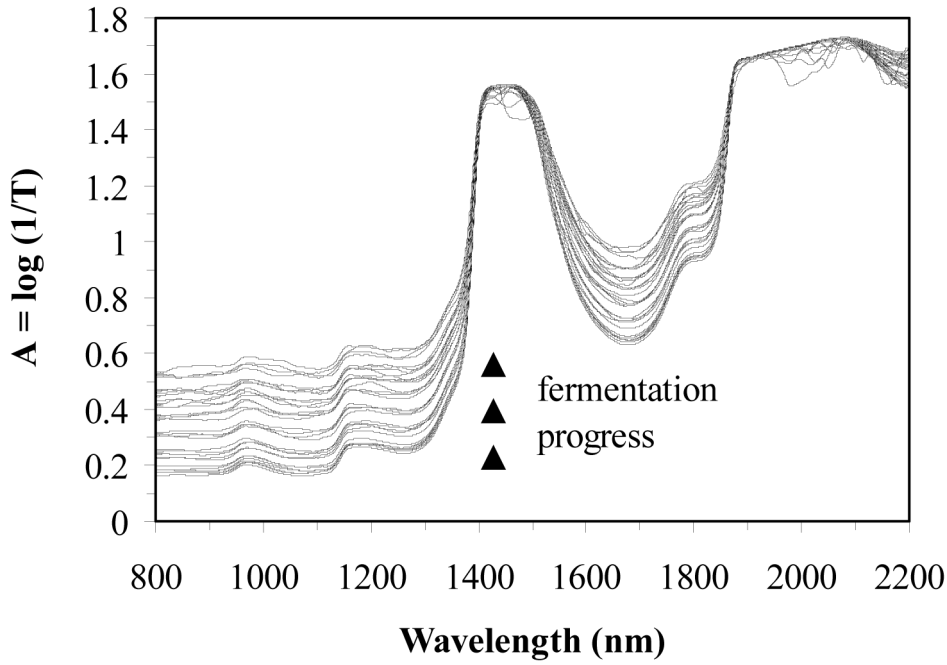

Figure 1. NIR spectra of glycerol-water mixture using 400 rpm, 600 rpm, 800 rpm, 1000 rpm, 1200 rpm, 1400 rpm, 1600 rpm and 2000 rpm of agitation in case of 270 degrees setting.

using 4 channels, equipped with interactance micro fiber optic bundles (3 m) with metal cladding and Ingold immersion probes with 2 mm optical pathlength. Samples were scanned (32 scans co-added) from 800 nm to 2199.5 nm (extended InGaAs detector). Data were collected at every 0.5 nm (2800 data points per spectrum). The raw spectra were transformed into first derivatives using 10/0 nm segment and gap size.[9]

Spectral data were processed using Vision 3.20 (Foss NIRSystems, Silver Spring, MD, USA) and Statistica 8.0 (Statsoft, Tulsa, OK, USA) softwares.

Results and discussion

A simplified medium was used in the first tests containing glycerol and water. The quantitative ratio of these materials was just the same as they were in the real fermentation broth. Aeration was kept at a constant level of 0.5 L min^{-1} but the revolutions per minute (rpm) of agitation was increased continuously as a function of oxygen demand of the broth. The effect of localisation of probe (0°, 90°, 180° and 270°) and of the level of agitation (400 rpm, 600 rpm, 800 rpm, 1000 rpm, 1200 rpm, 1400 rpm, 1600 rpm and 2000 rpm) were checked on NIR spectra. By increasing the rpm of agitation serious baseline shift occurred, caused by the air bubbles behaving as optical elements (Figure 1).

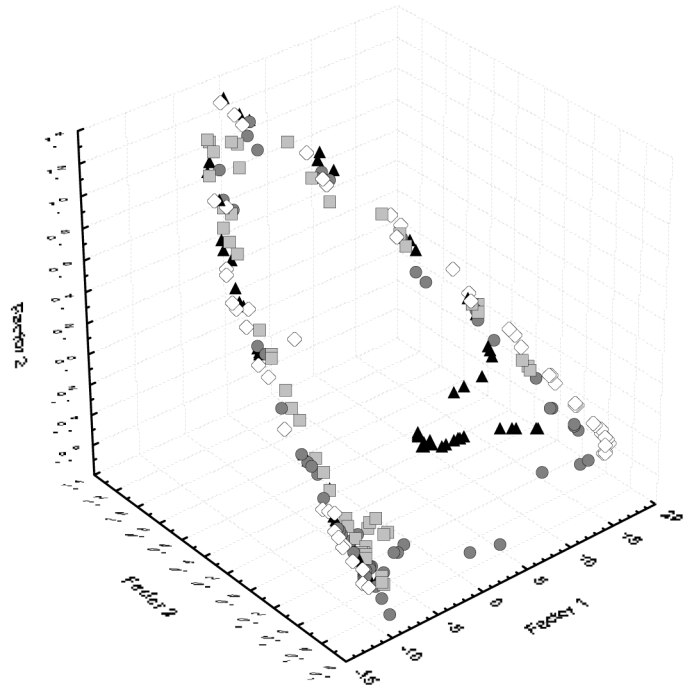

Figure 2. PCA scatter plots of scores for the first three principal components describing 99.68%, 0.30%, and 0.01 % of total variance from raw NIR spectra of four parallel experiments using the 800–1100 nm region at every 60th (± 5th) minutes.

At higher rpms higher absorbance levels were detected as a consequence of the lower amount of photons reaching the detector, because of the dispersion on the contact surfaces of medium and air bubbles. Hence some special protector elements were tested to eliminate the air bubble effect, and the most effective of them was chosen for further work.

Principal component analysis (PCA) was used for checking the similarity/differences between the spectral data files of parallel experiments. Water absorbs strongly in the NIR region, as seen in Figure 1.

The spectra appeared to be saturated in absorbance at 1400 nm to 1500 nm and 1850 nm to 2200 nm so the third and second overtones of NIR spectra were processed by PCA (Figure 2).

By running many parallel cultivations and analysing their NIR spectral data with PCA a so-called process fingerprint pattern can be derived for the particular process, which can give a lot of valuable information. For instance a batch failure could be detected at an early phase based on PCA process fingerprint pattern, which makes this method cost and time saving.

Calibrations were developed by the partial least squares (PLS) method for Ace, Gly, NH4+, and OD based on raw and first derivative spectra of four parallel experiments, after checking the cross-correlations of reference data. Different calibrations were processed in the case of Gly and Ace, depending on whether the whole dataset was used, or samples with Gly values under 2 g L^{-1} were ignored, or with Ace values equal to 0 mmol L^{-1} left out, assuming some uncertainty of the

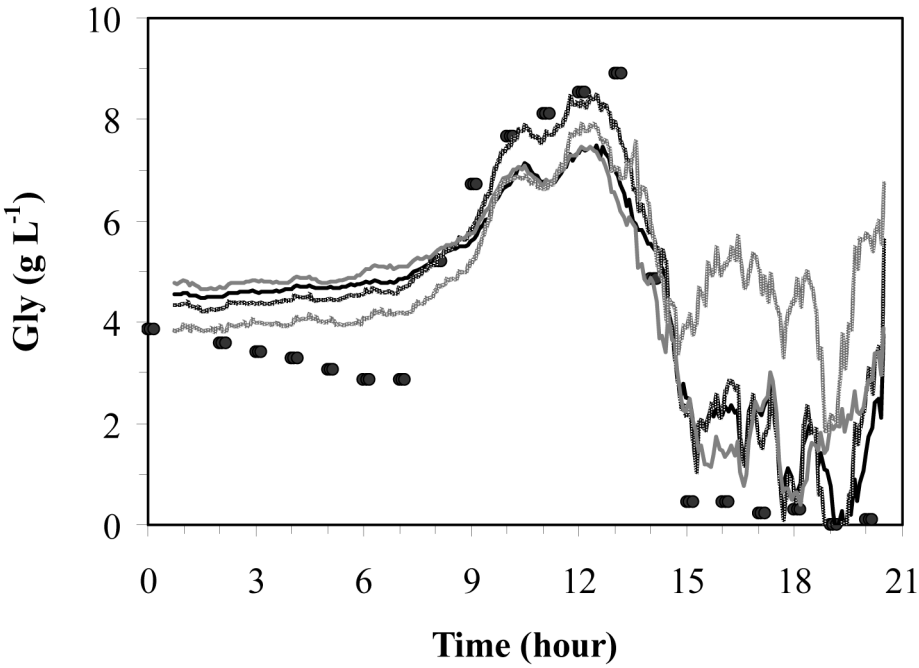

Figure 3. *E. coli* fermentation process accumulation profile for Gly (g L^{-1}); NIR predicted values are lines smoothing by ten-point moving averages, and reference chemical assays are solid symbols (●). Predictions were based on calibrations used both raw spectra (black lines) and first derivatives (grey lines), and both whole dataset (solid lines) and samples with Gly values under 2 g L^{-1} were ignored (pale lines).

reference method around these values. Predicted values were calculated with calibration equations to monitor the recent status of fermentation processes (Figures 3–4).

The results in case of Gly, Ace, and OD were acceptable to provide useful information about the critical changes of fermentation broths, but models for NH4+ showed a poor prediction capacity.

Improved models could be processed by developing the accuracy of reference methods, and increasing the number of parallel experiments, supported by further improvements in experimental design.

Conclusions

Qualitative observations and quantitative models were developed using PCA and PLS models in order to define the optimal conditions of fermentation, and to reduce the process variability from broth to broth. Application of NIR technology fits well into the PAT system, which will be implemented in the production of a biopharma product mentioned above.

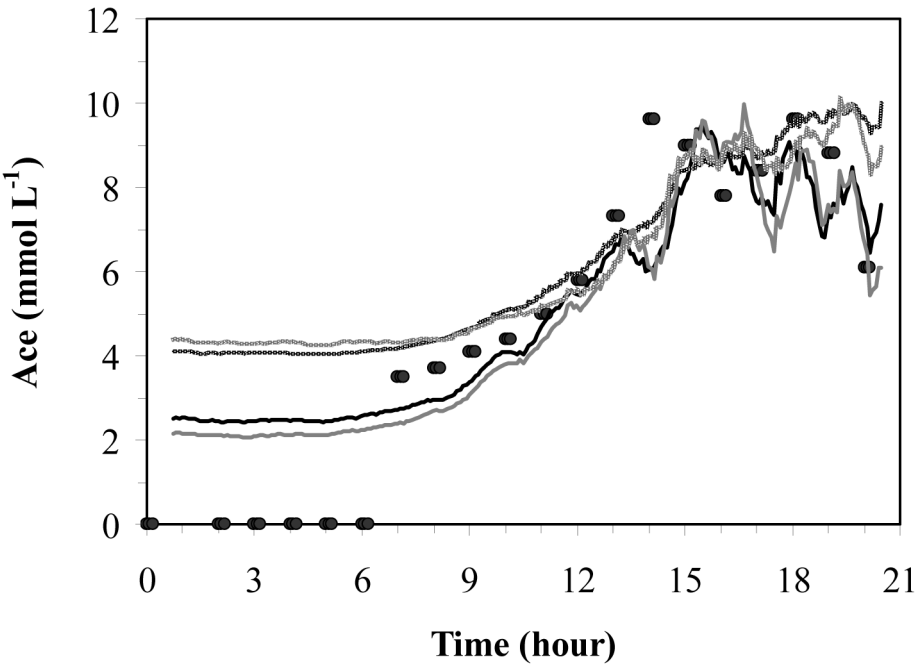

Figure 4. *E. coli* fermentation process accumulation profile for Ace (mmol L^{-1}); NIR predicted values are lines smoothing by ten-point moving averages, and reference chemical assays are solid symbols (●). Predictions were based on calibrations used both raw spectra (black lines) and first derivatives (grey lines), and whole dataset (solid lines) and samples with Ace values equal to 0 mmol L^{-1} were ignored (pale lines).

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