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Comparative study of non-invasive monitoring via infrared spectroscopy for mammalian cell cultivations

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ABSTRACT

Process analytical technology (PAT) is a guide to improve process development in biotech industry. Optical sensors such as near and mid infrared spectrometers fulfill an essential part for PAT. NIRS and MIRS were investigated as non-invasive on line monitoring tools for animal cell cultivations in order to predict critical process parameters, like cell parameters as well as substrate and metabolite concentrations. Eight cultivations were performed with frequent sampling. Variances between cultivations were induced by spiking experiments with intent to break correlations between analytes; to keep causality of the models; and to increase model robustness.

Calibration models were built for each analyte using partial least-squares regression method. Cultivations chosen for validation were not part of the calibration set. Glucose concentration, cell density and viability were predicted by NIRS with a root mean square error of prediction (RMSEP) of 0.36 g/L, 3.9 10^6 cells/mL and 3.62% respectively. Based on MIR spectra glucose and lactate concentrations were predicted with a RMSEP of 0.16 and 0.14 g/L respectively.

Results show that MIRS has higher accuracy regarding the prediction of single analytes. For prediction of a main course of a cultivation, NIRS is much better suited than MIRS.

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1. Introduction

In 2004 the process analytical technology (PAT) initiative was introduced by the US Food and Drug Administration (FDA). The intention was to encourage pharmaceutical companies to change their manufacturing procedure to a more science-based approach. Key elements of PAT are to reach a better understanding of processes, to identify critical process parameters (CPP) as well as critical quality attributes (CQA) and to implement real time process monitoring by multivariate data analysis tools (Rathore et al., 2010; Chew and Sharratt, 2010). The benefit from real time process monitoring is that problems can be detected early, and if possible, counteracted. Therefore it could be used for quality assurance or as an early fault detection system for the production of high-price products such as biologics. Taking into account that in 2012 the global sales of biologics was reported to be $US 120 billion, the possibility of reducing production costs and increasing efficiency would be of great economic benefit (Butler and Meneses-Acosta, 2012).

Most biologics are produced by animal cell cultures. Furthermore Chinese hamster ovary (CHO) cells are the most common animal cells in biotechnology. It is a very well characterized cell line and has been used for the production of more than 100 biopharmaceuticals (Butler and Meneses-Acosta, 2012). Global sales are estimated to increase by 25% by the year 2015. Reducing production costs through the use of proper online sensors systems which can fulfill PAT demands is a necessity.

Numerous inline sensors for measuring pH, temperature, dissolved oxygen, optical density, and exhaust gases are already available (Beutel and Henkel, 2011). However sensors for determination of CPPs and other critical variables such as nutrients or metabolites as well as cell parameters are still unavailable. Common cultivation practice involves taking samples up to twice a day and analyzing them offline by HPLC, GC or enzymatic assays. Those methods are invasive, time- and labor-consuming and therefore not suitable for real time process control depicted in the PAT initiative (Arnold et al., 2002). An ideal online sensor for PAT applications is non-invasive, delivers real time data and is capable of analyzing several parameters simultaneously.

Different optical sensors are available for bioprocess monitoring (Rehbock et al., 2008; Hantelmann et al., 2006; Bluma et al., 2011). Especially near and mid infrared spectroscopy (NIRS and MIRS) have great potential in bioprocess monitoring. An extensive review about many applications involving NIRS and MIRS can be found here (Landgrebe et al., 2010; Haake et al., 2009). They can be applied in situ by free beam or fiber optics, are non-invasive and provide

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real time process information. In combination with multivariate data analysis, multiple CPPs can be determined simultaneously.

A general approach for MIRS and NIRS is to use fiber optic immersive probes which can be attached to the bioreactor through standard ports. In bioprocess technology flow-through cells are not feasible for MIRS because they require optical path lengths of less than 0.1 mm (Hannah, 2002). A common sampling technique for MIRS in aqueous solutions is based on attenuated total reflection (ATR) (Harrick, 1967). Silver halide fibers transmit IR light from the spectrometer to the diamond tip of an ATR into the liquid with a penetration depth of typically 2 μm. Due to the high self-absorbance of the fiber material, the length of the optical fiber is usually limited to two meters. MIR spectra consist of excitations of fundamental vibrations in the so-called “fingerprint” region between 1500 and 500 cm^{-1} (Landgrebe et al., 2010). This region is used to identify chemical compounds due to their specific absorption pattern.

The standard sampling technique for NIR consists of immersive flow through probes operating either in reflectance or transflectance with an optical path length between 1 and 2 mm (Henriques et al., 2010). Compared to MIRS, probe and spectrometer are connected by fused silica fibers (Arnold et al., 2002). These fibers have a low self-absorbance in the NIR region and are considerably less expensive which introduces the possibility of remote sampling (Henriques et al., 2010). An alternative to fiber optic probes is the use of a NIR spectrometer equipped with free beam optics in combination with diode array detectors. In this case the probe is directly connected to the spectrometer without any fibers. This requires a compact, and robustly built spectrometer which can be operated in harsh environments.

Observed NIR spectra consist of combinations and overtones of fundamental vibrations (Landgrebe et al., 2010). Absorption bands are much broader, and absorption coefficients are lower than in MIRS. Due to a larger optical path length and the transflection geometry, NIRS is affected by light scattering that is caused by small particles such as animal cells. Resulting changes in NIR spectra enable real time monitoring of cell parameters.

In biotechnology, culture media contains many different compounds resulting in very complex MIR and NIR spectra. Extracting the essential information out of the spectra caused by a specific analyte is accomplished by multivariate data analysis tools. Especially in the NIR region with broad and overlapping absorption bands, this approach is crucial for data evaluation (Schenk et al., 2007; Henriques et al., 2010). For quantitative analysis the standard multivariate tool is PLS regression (Henriques et al., 2010). The PLS algorithm correlates spectral variations with analyte concentrations obtained from offline measurements to generate a calibration model (Gemperline, 2006; Henriques et al., 2010). The calibration yields a PLS model which can be used for prediction of analyte concentration based on spectral data only.

One aim of this study is to investigate MIR and NIR spectroscopy as online monitoring tools for CHO cell cultivations as a PAT solution. As correlations between analytes are a common problem in PLS analysis this problem is addressed, and solved, by spiking experiments. In addition, by artificially inducing variances between cultivation models which are then tested in regard to their robustness and validated externally.

2. Materials and methods

2.1. Cultivation process

A CHO-K1 cell line (Cell Culture Technology, University of Bielefeld, Germany) is cultivated in a commercially available serum-free medium TC-42 (TeutoCell AG, Bielefeld, Germany). The culture media contains 7.59 g/L glucose and is free of hypoxanthine, thymidine and glutamine. In order to avoid the slightest differences in the media composition, culture media from a single batch is employed. To promote cell growth TC-42 is supplemented with glutamine (100 mM) to a total concentration of 8 mM. Since glutamine decomposes even if stored at 4 °C it is thawed and added shortly before inoculation (Tritsch and Moore, 1962).

Cultivations are carried out in a 10-L bioreactor (Biostat® Cplus, Sartorius Stedim Biotech GmbH, Göttingen, Germany) with a working volume of 7.5 L. Inocula for the reactor cultivation are grown in shake flasks (37 °C, 150 rpm, 5% CO_2) in three steps. The seeding starts from 1 mL stock cultures with a cell density of 1×10^7 cells/mL, thawed in 24 mL media and cultivated for 96 h in 100 mL shake flasks. Every further passage is inoculated with a cell density of 4×10^5 cells/mL. The second passage is carried out at a total volume of 30 mL and cultivated under the same conditions. The third, and last, passage is performed in four 250 mL shake flasks to expand and produce a sufficient cell number before the reactor inoculation, again for 96 h. Depending on the final cell density of the starting culture, approximately 330 mL of the last passage are needed to inoculate the 10-L reactor with a final cell concentration of 4×10^5 cells/mL.

Bioreactor cultivations are performed under controlled conditions. Temperature is maintained at 37 °C. The pH is monitored by an electrochemical electrode (Easy Ferm Plus K8, Hamilton Messtechnik GmbH, Höchst, Germany) and fixed at 7.1 by automatic addition of 1 M Na_2CO_3 or by aeration with CO_2. To minimize the risk of contamination and to increase the oxygen intake the bioreactor is pressurized (0.1 bar). Antifoam (Pluronic® F68, 4 mL 10% solution per liter TC-42) is supplemented to prevent an excess formation of foam during the exponential phase.

Dissolved oxygen is monitored via an amperometric electrode (Oxyferm FDA, Hamilton Messtechnik GmbH, Höchst, Germany). It is maintained at 40% by automatic aeration via ring sparger with an air or air-oxygen mixture. In order to keep foam formation and mechanical stress for the cells at a minimum, the maximum aeration rate is limited to 0.5 L/min for the first four cultivations and 0.3 L/min for the remaining four. Changing the maximum aeration is done to increase process variability. The stirring rate is fixed at 200 rpm.

A total of eight cultivations were executed. Three are performed as batch cultivations and the other five cultivations are fed to spike the media with glucose and glutamine. These will be referred to as spiking experiments and they are done to break correlations between analytes and cell parameters and to increase process variability. A glucose solution (300 g/L) is used for all fed-batch cultivations as a basic feed to spike the media with glucose. Glutamine (100 mM) is also added during one of the fed-batch cultivations prior to the glucose feeding.

Three different feeding strategies for glucose are conducted. In one cultivation, glucose is added after it is consumed in three steps up to a final concentration of 9 g/L. For the remaining experiments glucose is kept at two different concentration levels (1–2 g/L and 2–4 g/L) before it is depleted. Glutamine is added during a single cultivation before it is completely consumed in two steps to a maximum concentration of 8 mM.

2.2. Sampling and reference analysis

All reference assays are conducted in triplicate and all chemicals (VWR International GmbH, Darmstadt, Germany) used for analysis are of pharmaceutical grade. Samples are collected through a steam sterilizable sample port every 3 h. Overnight, the interval is reduced to every 6 h and during spiking experiments, increased to every hour in order to capture critical changes. All analyzed parameters including their observed ranges are listed in Table 1.

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