Talanta 111 (2013) 28–38

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Chemometrics and in-line near infrared spectroscopic monitoring of a biopharmaceutical Chinese hamster ovary cell culture: Prediction of multiple cultivation variables

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ARTICLE INFO

Article history:
Received 18 December 2012
Received in revised form
14 March 2013
Accepted 16 March 2013
Available online 26 March 2013

Keywords:
Chinese hamster ovary
Near infrared spectroscopy
Cell culture
Bioprocess monitoring
Process analytical technology
Chemometrics

ABSTRACT

In the present study near infrared (NIR) spectroscopy was used to monitor the cultivation of mammalian Chinese hamster ovary (CHO) cells producing a monoclonal antibody in a fed-batch cell culture process. A temperature shift was applied during the cultivation. The cells were incubated at 37 °C and 33 °C. The Fourier transform near infrared (FT-NIR) multiplex process analyzer spectroscopy was investigated to monitor cultivation variables of the CHO cell culture from 10 independent batches using two channels of the FT-NIR. The measurements were performed on production scale bioreactors of 12,500 L. The cell cultures were analyzed with the spectrometer coupled to a transflection sterilizable fiber optic probe inserted into the bioreactors. Multivariate data analysis (MVDA) employing unsupervised principal component analysis (PCA) and partial least squares regression methods (PLS) were applied. PCA demonstrated that 96% of the observed variability was explained by the process trajectory and the inter-batch variability. PCA was found to be a significant tool in identifying batch homogeneity between lots and in detecting abnormal fermentation runs. Seven different cell culture parameters such as osmolality, glucose concentration, product titer, packed cell volume (PCV), integrated viable packed cell volume (ivPCV), viable cell density (VCD), and integrated viable cell count (iVCC) were monitored inline and predicted by NIR. NIR spectra and reference analytics data were computed using control charts to evaluate the monitoring abilities. Control charts of each media component were under control by NIR spectroscopy. The PLS calibration plots offered accurate predictive capabilities for each media. This paper underlines the capability for inline prediction of multiple cultivation variables during bioprocess monitoring.

1. Introduction

Pharmaceutical and biotechnological companies are encouraged to optimize the efficiency of their established processes. For economic, technical, regulatory and quality requirements the optimization of biotechnological processes is essential. It is under these conditions that the food and drug administration (FDA) encourages pharmaceutical firms to set up innovative tools to better understand their processes. For this purpose the FDA has launched a regulatory framework initiative called process analytical technologies (PAT) [1]. In parallel, the international conference of harmonisation (ICH) has suggested that all critical process parameter which act upon critical quality attribute should be monitored or controlled according to the ICH Q8(R2) "Pharmaceutical Development" guideline [2]. In this way, real time process monitoring becomes interesting, to optimize productivity (by controlling, understanding, troubleshooting bioprocesses) and to ensure product quality. Thus, vibrational spectroscopy has emerged as one of the key analytical tools to be used and traditional wet-chemistry analyses tend to be replaced by fast estimated analytics.

Vibrational spectroscopy covers near infrared (NIR), mid-infrared (MIR) and Raman spectroscopy. These tools are suitable for solid, liquid and biotechnological pharmaceutical forms. In this study NIR was used. NIR can be implemented during pharmaceutical development phases, in production for process monitoring or in quality control laboratories for release and stability analysis [3]. NIR is a fast and non-destructive vibrational spectroscopy based on molecular overtone and combination vibrations. The molecular broad bands seen in the NIR lead to more or less complex spectra. In NIR it is difficult to assign specific features to specific chemical components. NIR delivers chemical and physical information. Thus, multivariate calibration algorithm coupled with statistical methods (e.g. Multivariate data analysis or chemometrics) are

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http://dx.doi.org/10.1016/j.talanta.2013.03.044

used to create qualitative and quantitative models by extracting the desired information [4,5].

The objective of this study is to apply NIR spectroscopy on a biopharmaceutical product to monitor in-line Chinese hamster ovary (CHO) cell culture and bring new features for quantification of multiple components. NIR spectroscopy has been used to monitor a large number of bioprocesses and not only related to biopharmaceutical products. The aim here is not to present all papers which successfully used these techniques but to show features already done as the basis of our paper. Recently, the application of at-line NIR transmittance spectroscopy on supernatant samples from CHO cells producing a monoclonal antibody has been published [6]. It was demonstrated that parameters of interest like nutrient or product concentrations can be reliably estimated with at least an accuracy of 15% versus the reference method. Moreover, it was concluded that NIR monitoring carried out at-line was effective in replacing in-process monitoring of some critical process parameters by reference methods. Previously, Strother, *T.* has predicted by NIR CHO protein concentrations in a live and dynamic cell culture [7]. A Thermo® Process analyzer was able to predict protein to 0.5 g L^{-1} on a wide range from 0.16 to 5.23 g L^{-1}. In 2008, NIR was used to monitor multiple components in mammalian cell culture of Human Embryonic cells [8]. The authors were able to develop PLS calibration for cell density, pH, and lactate, glucose, ammonia and glutamine concentration. In 2007, a proposal to build NIR calibrations from multiple bioreactors run in parallel was published [9]. The authors observed a slight model degradation in comparison to the conventional single probe models. In 2003, Arnold and Al published results on the use of in-situ NIR to monitor analytes in a CHO-K1 culture (derived as a subclone from the parental CHO) [10]. They were able to develop models for glucose, lactate, glutamine, and ammonia concentration with interesting standard error of prediction results. In 1998, NIR was used to measure the concentration of human antithrombin III and metabolites in a CHO cell culture supernatant [11]. All these papers show that the monitoring of multiple components on cell culture is possible but with differing results. In our study we will apply NIR on a large production scale.

The calibration strategy developed in this paper is presented in Fig. 1. During production runs, offline data are generated to observe culture conditions. By connecting a NIR spectrometer to the production run, spectral information is produced. The aim was to correlate the spectral information with the offline data in order to propose statistical calibration models for inline monitoring.

2. Materials and methods

2.1. Cell culture conditions and detail

In an aerobic cell culture process, CHO cells producing a monoclonal antibody were cultivated in large scale bioreactors. The culture duration was approximately 15 days. During the cultivation a temperature shift was performed on day three from 37 °C to 33 °C until the end. 10 independent batches were monitored.

2.2. Near infrared acquisition

The 10 cell culture batches were monitored using a Fourier transform near infrared (FT-NIR) multiplex process analyzer (Antaris II MX, Thermo Fisher Scientific®, Madison, USA) qualified to be used under good manufacturing practice (GMP). The spectrometer was programmed to take spectra continuously during the whole culture process. The spectrometer was equipped with a sterilizable transflection probe (Helma®, Type 661.687, Germany) connected to fiber optics. The probe with a pathlength distance of 1 mm was inserted into the bioreactor and sterilized prior to running the cell culture process. 256 scans were averaged per spectrum using a 8 cm^{-1} resolution on a spectral range from 4000 to 10,000 cm^{-1}. A delay of 240 s was applied between 2 spectra. Sample spectra were corrected by using the background transfer function TS/TB. This function addresses the difference between background path (intern standard called TB) and sample path (air spectrum called TS). This sample spectra correction is specified by (S/B)/(TS/TB) where (TS/TB) is the background transfer function, S is the sample spectrum (media spectrum) and B the background spectrum (intern standard) [12].

Each batch was measured independently. Two channels and two probes of the same type were used to acquire spectra from two bioreactors in parallel. The chemometric models were developed by computing 3 spectra for each reference value.

2.3. Wet chemical analysis used as reference

All reference data were sampled twice per day during the whole cell culture. The cell culture performance in each bioreactor was monitored through in-line and off-line reference data measurements.

The Bioprofile 400 (Nova Biomedical Corporation®, Massachusetts, United States) was used to determine the glucose concentration in g L^{-1}. The measurement of osmolality expressed in mOsm was

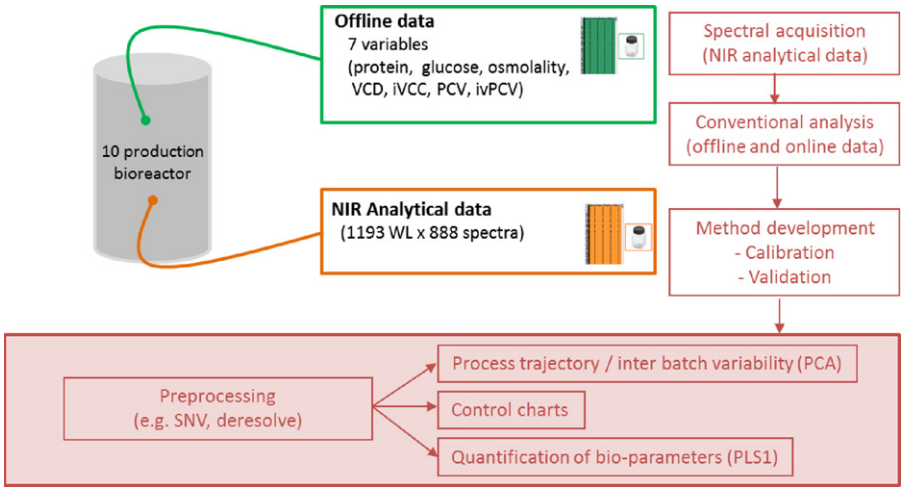

Fig. 1. Calibration strategy applied on the 10 bio-processes runs.